IMMUNOGENIC COMPOSITIONS FOR *CHLAMYDIA PNEUNOMIAE*

This application claims the benefit of U.S. Provisional Application 60/542,832, filed March 2, 2004; U.S. Provisional Application 60/643,110, filed January 12, 2005; and U.S. Provisional Application 60/644,552, filed January 19, 2005, all of which are *incorporated herein in their entireties.*

All documents cited herein are incorporated by reference in their entirety.

Field

The invention is in the field of immunology and vaccinology. In particular, it relates to immunogenic compositions comprising combinations of immunogenic molecules from *Chlamydia pneumoniae.*

Background Art

The bacteria of the genus *Chlamydia* (and *Chlamydophila*, according to the recently proposed but still controversial re-classification of *Chlamydia*ceae (Bush *et al* (2001) Int J Syst Evol Microbiol 51: 203-20; Everett *et al* (1999) Int J Syst Bacteriol 49: Pt2 415-40; Schachter *et al* (2001) Int J Syst Evol Microbiol 51: 249, 251-3) are obligate intracellular parasites of eukaryotic cells, which have a unique biphasic life cycle involving two pleiomorphic developmental forms: an extracellular, metabolically inert, spore-like, infectious form (the elementary bodies, EBs) and an intracellular, non-infectious, replicative form (the reticulate bodies, RBs) which remains contained in a specialized cytoplasmic compartment (the *Chlamydia*l inclusion). The EBs are responsible for the initial attachment to host cell surface and the establishment of the cytoplasmic inclusion where EBs can differentiate to RBs and thus initiate the replicative stage. Eventually RBs revert to infectious EB forms able to start new replicative cycles in neighbouring host cells.

As *Chlamydia* infection is an intracellular infection, the currently accepted paradigm is that effective anti-*Chlamydia*l immunisation would require both an adequate T-cell response and high serum levels of neutralising antibodies and that "an ideal vaccine should induce long lasting (neutralising) antibodies and a cell mediated immunity that can quickly respond upon exposure to *Chlamydia*". Several sometimes contradictory studies have indicated that both CD4+ and CD 8 positive T cells have a role in *Chlamydia*l clearance (Loomis and Starnback (2002) Curr Opin Microbiol 5: 87-91). Indeed, there now appears to be a prevailing consensus that specific CD4+ T cells and B cells are critical to the complete clearance of intracellular *Chlamydia* and for mediating recall immunity to *Chlamydia* infection (see Igietseme, Black and Caldwell (2002) Biodrugs 16: 19-35 and Igietseme *et al* (1999) Immunology 98: 510-519).

Whilst it is now possible to carry out searches of the whole *Chlamydia pneumoniae genome, there is still insufficient information available on parallel proteome* characterisation. By way of example, while sequence data is available for many of the *Chlamydia pneumoniae* antigens, there is insufficient characterisation of the *Chlamydia* antigens in terms of their immunological and/or biological function. By way of example, whilst applications such as WO 99/28475 and WO 99/27105 disclose sequence information, there is no characterisation of these sequences in terms of their immunological and/or biological function. In contrast, WO 02/02404 provides information on the immunogenicity and immunoaccessibility of certain

Chlamydia proteins and highlights that (i) current genomic annotations and/or (ii) predictions based on cellular location and/or cellular function based on *in-silico* analyses may not always be accurate.

Applicants have recently engaged in a whole-genome search (Montigiani *et al* (2002) Infection and Immunity 70:368-379) for possible vaccine candidates among proteins potentially associated with the outer membrane of *C.pneumoniae*. For this study, mouse antisera was prepared against over 100 recombinant His-tagged or Glutathione-S-transferase (GST) fusion proteins encoded by genes predicted by in silico analyses to be peripherally located in the *Chlamydia*l cell. From this screening study, 53 recombinant proteins derived from the genome of *Chlamydia (Chlamydophila pneumoniae* (CPn) were described which induced mouse antibodies, capable of binding, in a FACS assay, to the surface of purified CPn cells.

The scope of the Montigiani study (*ibid*) was restricted to checking if polyclonal antisera produced in mice against the recombinantly expressed antibodies to CPn antigens were capable of binding to the surface of the CPn cells. No studies were carried out to test whether antisera against the recombinant FACS positive antigens were capable of interfering with EB *in vitro* infectivity of host cells – that is, whether the murine antibodies raised against the recombinantly expressed antigens could inhibit CPn infectivity *in vitro* to an extent greater than 50%, a property that common practice qualifies such antigens as "neutralising".

Indeed, so far, only few *C. pneumoniae* antigens with 'neutralizing' properties have been described in the literature: notably, a protein identified as 76-kDa-homolog protein (Perez-Melgosa *et al* (1994) Infect Immunity 62: 880-6), the surface-exposed outer membrane proteins MOMP (Wolf *et al* (2001) Infect Immun 69: 3082-91), PorB (Kawa *et al* (2002) J Immunol 168 : 5184-91 and Kubo *et al* (2000) Mol Microbiol 38 : 772-80), and very recently also the Pmp21 member of the *Chlamydia*-specific polymorphic family of outer membrane proteins (A.Szczepek, personal comunication). All these proteins were in fact selected in the earlier FACS-based screening study (Montigiani *et al* (2002) *ibid*). It can be however noted that outer membrane antigens, as it is the case for MOMP and PorB, could possibly present some kind of practical problems for a recombinant vaccine development project. For instance both MOMP and PorB are integral membrane proteins which appear to require a native conformation to maintain neutralizing epitopes which are discontinuous and conformation-dependent. The production of such proteins may require special processing steps (refolding) which could be undesirable in the preparation of an hypothetical vaccine. Other general problems may arise from the extent of allelic variation, and from regulated proteins which are not always expressed in all *Chlamydia*l cell or all *Chlamydia*l isolates.

Thus, it is desirable to provide improved compositions capable of eliciting an immune response upon exposure to *Chlamydia pneumoniae* proteins. It is also desirable to provide improved compositions comprising one or more combinations of two or more selected CPn proteins with complementary immunological and/or biological profiles capable of providing immunity against *Chlamydia*l induced disease and/or infection (such as in prophylactic vaccination) or (b) for the eradication of an established chronic *Chlamydia*l infection (such as in therapeutic vaccination).

Brief description of the drawings and tables

Figure 1A. Assay of *in vitro* neutralization of *C.pneumoniae* infectivity for LLC-MK2 cells by polyclonal mouse antisera to recombinant *Chlamydia*l proteins.

Figure 1B shows serum titres giving 50% neutralization of infectivity for 10 *C.pneumoniae* recombinant antigens. Each titer was assessed in 3 separate experiments (SEM values shown).

Figure 2 shows immunoblot analysis of two dimensional electrophoretic maps of *C.pneumoniae* EBs using the imune sera described in the text.

Figure 3 shows mean numbers of *C.pneumoniae* IFU recovered from equivalent spleen samples from immunized and mock-immunized hamsters following a systemic challenge.

Figure 4 shows flow cytometric analysis of splenocytes from DNA-immunized HLA-A2 transgenic and non transgenic mice.

Figure 5 shows a flow cytometric analysis of splenocytes from transgenic and non transgenic mice infected with *C. pneumoniae* EBs.

Figure 6 shows an alignment of the proteins in the 7105-7110 protein family.

Figure 7 shows an N-terminal alignment of Cpn0794 – Cpn0799.

Figure 8 shows a protein encoded by Cpn0796 and demonstrates a C-terminal domain comprising approximately residues from 1 to 648.

Figure 9 shows an alignment of the C-terminal (beta barrel) domains of the proteins encoded by the C.pneumoniae genes Cpn0795 and Cpn0796.

Table I shows a summary of data and properties of the *C.pneumoniae* antigens described in the text.

Table 2 shows results from hamster mouse model studies for hypothetical proteins.

Table 3 shows expressed genes of CPn EB selected by microarray.

Table 4 shows *C. pneumoniae* selected peptides: protein sources and HLA-A2 stabilization assay.

Table 5 shows ELISPOT assay with CD8+ T cells from DNA immunised HLA-A2 transgenic mice.

Table 6 shows IFN-γ production from splenocytes of DNA immunized HLA-A2 transgenic and non transgenic mice.

Summary of the Invention

The present invention relates to a polypeptide for use as an autotransporter antigen, the polypeptide comprising: (a) an amino acid sequence selected from the group consisting of SEQ ID NO: 54, SEQ ID NO: 6, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 78, and SEQ ID NO: 79, (b) an amino acid sequence having at least 50% sequence identity to an amino acid sequence of (a); or (c) an amino acid sequence comprising one or more fragments of at least 7 consecutive amino acids from an amino acid sequence of (a) or combinations thereof.

The present invention also relates to the use of a polypeptide in the preparation of a medicament for the prevention or treatment of a Chlamydia pneumoniae infection in an individual. For example, the use of the polypeptide may be as an autotransporter protein which immunoreacts with seropositive serum of an individual infected with Chlamydia pneumoniae.

The present invention further relates to a method of eliciting an immune response in an individual comprising administering to the individual a polypeptide comprising (a) an amino acid sequence selected from the group consisting of SEQ ID NO: 54, SEQ ID NO: 6, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 78, and SEQ ID NO: 79 (b) an amino acid sequence having at least 50% sequence identity to an amino acid sequence of (a), or (c) an amino acid sequence comprising one or more fragment of at least 1, 2, 3, 4, 5, 6, or 7 amino acids from an amino acid sequence of (a) or mixtures thereof.

Also, a method is provided for diagnosing an immune response in an individual comprising (a) contacting a biological sample obtained from the individual with a binding agent that binds to a polypeptide with an autotransporter function, (b) detecting in the biological sample the amount of the polypeptide that binds to the binding agent; and (c) comparing the amount of the polypeptide to a predetermined cut-off value and thereby determining the presence of an immune response in the individual.

A composition for eliciting an immune response in a subject comprising two or more Chlamydia pneunoniae autotransporter proteins or immunogenic fragments thereof is also provided. The composition may further comprise one or more immunostimulants.

Also provided is a polypeptide for use as an autotransporter antigen comprising an amino acid sequence corresponding to SEQ ID NO: 86, an amino acid sequence having at least 50% sequence identity to SEQ ID NO: 86, or an amino acid sequence comprising one or more fragments of at least 7 consecutive amino acids of SEQ ID NO: 86.

The present invention relates to a composition comprising a first biological molecule from a *Chlamydia pneumoniae* bacterium and a second biological molecule from a *Chlamydia pneumoniae* bacterium. The first biological molecule is selected from the group consisting of SEQ ID No 1 to SEQ ID No 86, or the group consisting of SEQ ID No. 1 to 41.

The composition may also contain the second biological molecule being selected from the group consisting of SEQ ID No 1 to SEQ ID No. 86 or SEQ ID No 1 to SEQ ID No 41.

The composition may also comprise two or more biological molecules selected from the group consisting of SEQ ID Nos 1-41.

The composition may also comprise one or more biological molecules selected from the group consisting of SEQ ID Nos 1-41 combined with one or more biological molecules selected from the group consisting of SEQ ID Nos 42-86.

The composition according to any one of the previous claims further comprising an adjuvant such as an ADP-ribosylating exotoxin or a derivative thereof or an adjuvant is selected from the group consisting of cholera toxin (CT), Escherichia heat-labile exterotoxin (LT) and mutants thereof having adjuvant activity.

A vaccine and use of the vaccine is also provided comprising the composition of the present invention. The vaccine may be used in the preparation of a medicament for the prevention or treatment of a *Chlamydia* infection and may be administered mucosally, intra-nasally or intra-vaginally, for example.

Further, a method is provided for treating a *Chlamydia* infection in a host subject wherein the method comprises the administration of a safe and effective amount of a vaccine.

In another aspect of the invention, an immunogenic composition is provided comprising a combination of *Chlamydia pneumoniae* antigens, the combination comprising at least one *Chlamydia pneumoniae* antigen associated with elementary bodies of *Chlamydia pneumoniae* and at least one *Chlamydia pneumoniae* antigen associated with reticulate bodies of *Chlamydia pneumoniae*.

In another aspect of the invention, an immunogenic composition is provided comprising a combination of *Chlamydia pneumoniae* antigens, the combination comprising at least one *Chlamydia pneumoniae* antigen of a first antigen group and at least one *Chlamydia pneumoniae* antigen of a second antigen group, said first antigen group comprising a Type III secretion system (TTSS) protein and said second antigen group comprising a Type III secretion system (TTSS) effector protein.

In yet another aspect of the invention, an immunogenic composition is provided comprising a combination of *Chlamydia pneumoniae* antigens comprising at least one *Chlamydia pneumoniae* antigen that is conserved over at least two serovars.

In still another aspect of the invention, an immunogenic composition is provided comprising a combination of *Chlamydia pneumoniae* antigens, the combination eliciting a *Chlamydia pneumoniae* specific TH1 immune response and a *Chlamydia pneumoniae* specific TH2 immune response.

The present invention further provides a method of monitoring the efficacy of treatment of a patient infected with *Chlamydia pneumoniae* comprising determining the level of *Chlamydia pneumoniae* specific antibody in the patient after administration of an immunogenic composition of the present invention to the patient.

Description of the Invention

The present invention provides compositions comprising a first biological molecule from a *Chlamydia pneumoniae* bacterium and a second biological molecule from a *Chlamydia pneumoniae* bacterium. The term "biological molecule" includes proteins, antigens and nucleic acids. The compositions may also comprise further biological molecules preferably also from *Chlamydia pneumoniae*. That is to say, the compositions may comprise two or more biological molecules (eg. 3, 4, 5, 6, 7, 8 etc.) at least two of which are from a *Chlamydia pneumoniae* bacterium (eg. 3, 4, 5, 6, 7, 8 etc.). Such compositions include those comprising (i) two or more different *Chlamydia pneumoniae* proteins; (ii) two or more different *Chlamydia pneumoniae* nucleic acids, or (iii) mixtures of one or more *Chlamydia pneumoniae* protein and one or more Chlamydia pneumoniae nucleic acid.

In one aspect of the present invention, an immunogenic composition is provided comprising a combination of at least one antigen that elicits a *Chlamydia pneumoniae* specific TH1 immune response (such as a cell mediated or cellular immune response) and at least one antigen that elicits a *Chlamydia pneumoniae* specific TH2 response (such as a humoral or antibody response). The immunogenic composition may further comprise a TH1 adjuvant and a TH2 adjuvant.

In another aspect of the present invention, an immunogenic composition is provided comprising a combination of *Chlamydia pneumoniae* antigens comprising at least one *Chlamydia pneumoniae* antigen that is conserved over at least two serovars.

In yet another aspect of the present invention, an immunogenic composition is provided comprising a combination of at least one antigen that elicits a *Chlamydia pneumoniae* specific TH1 immune response and at least one antigen that elicits a *Chlamydia pneumoniae* specific TH2 immune response, the combination comprising at least one *Chlamydia pneumoniae* antigen that is conserved over at least two serovars.

In another aspect of the present invention, the immunogenic composition comprising at least one antigen that elicits a *Chlamydia pneumoniae* specific TH1 immune response and at least one antigen that elicits a *Chlamydia pneumoniae* specific TH2 immmune response preferably comprises a combination of *Chlamydia pneumoniae* antigens comprising at least one *Chlamydia pneumoniae* antigen associated with the EB of *Chlamydia pneumoniae* and at least one *Chlamydia pneumoniae* antigen associated with the RB of *Chlamydia pneumoniae*. Still further such combinations can comprise EB and/or RB antigens from one serovar combined with RB and/or EB antigens from at least one other serovar.

In an additional aspect of the present invention, a kit is provided comprising a combination of *Chlamydia pneumoniae* antigens wherein at least one of the *Chlamydia pneumoniae* antigens is associated with the EB of *Chlamydia pneumoniae* and at least one of the *Chlamydia pneumoniae* antigens is associated with the RB of *Chlamydia pneumoniae*. The kit may further include a TH1 adjuvant, a TH2 adjuvant and instructions.

The present invention further provides methods of eliciting a Chlamydia specific immune response by administering an immunogenic composition of this invention.

The present invention further provides a method of monitoring the efficacy of treatment of a subject infected with *Chlamydia pneumoniae* comprising determining the level of Chlamydia specific antibody or Chlamydia specific effector molecule in the subject after administration of an immunogenic composition of this invention.

In one preferred embodiment the first and second biological molecules are from different *Chlamydia pneumoniae* species (for example, from different *Chlamydia pneumoniae* serovars) but they may be from the same species. The biological molecules in the compositions may be from different serogroups or strains of the same species. The first biological molecule is preferably selected from the group consisting of SEQ ID Nos 1-86. More preferably. it is selected from the group consisting of SEQ IDs 1-41and/or SEQ ID Nos 42-86. It is preferably a purified or isolated biological molecule. The second biological molecule is preferably selected from the group consisting of SEQ ID Nos 1-86. More preferably. it is selected from the group consisting of SEQ IDs 1-41 and/or SEQ ID Nos 42-86. It is preferably a purified or isolated biological molecule. Specific compositions according to the invention therefore include those comprising: two or more biological molecules selected from the group consisting of SEQ ID Nos 1-41; one or more biological molecules selected from the group consisting of SEQ IDs 1-41 combined with one or more biological molecules selected from the group consisting of SEQ IDs 42-86. One or both of the first and second biological molecules may be a *Chlamydia pneumoniae* biological molecule which is not specifically disclosed herein, and which may not have been identified, discovered or made available to the public or purified before this patent application was filed.

In another embodiment, a combination of *Chlamydia pneumoniae* antigens is provided, the combination comprising at least one Type III Secretion System (TTSS) protein and at least one Type III Secretion System (TTSS) secreted or effector protein or fragment thereof. There are many methods for identifying TTSS proteins (i.e., TTSS proteins associated with the Chlamydial TTSS machinery). TTSS is a complex protein secretion and delivery machine or apparatus, which may be located, either wholly or partially, on the Elementary Body (EB) and which allows an organism, such as Chlamydia, to maintain its intracellular niche by injecting proteins, such as bacterial effector proteins (which may act as anti-host virulence determinants) into the cytosol of a eukaryotic cell in order to establish the bacterial infection and to modulate the host cellular functions. TTSS proteins exposed on the EB surface may play a role in adhesion and/or uptake into host cells.

By way of background information, the TTSS is a complex protein secretion and delivery machine or apparatus, which may be located on the Elementary Body (EB) and which allows an organism, such as Chlamydia, to maintain its intracellular niche by injecting proteins, such as bacterial effector proteins (which may act as anti-host virulence determinants) into the cytosol of a eukaryotic cell in order to establish the bacterial infection and to modulate the host cellular functions. These injected proteins (the TTSS effector proteins) can have various effects on the host cell which include but are not limited to manipulating actin and other structural proteins and modification of host cell signal transduction systems. The injected (or translocated) proteins or substrates of the TTTS system may also be processed and presented by MHC-class I molecules.

Not all the proteins secreted by a Type III secretion system are delivered into the host cell or have effector function. Although the Elementary Body (EB) is regarded as "metabolically inert", it has been postulated that the Chlamydial TTSS system located on the (EB) is triggered by membrane contact and is capable of releasing pre-formed "payload" proteins. The current hypothesis is that Type Three Secretion System (TTSS) becomes active during the intracellular phase of the chlamydial replicative cycle for the secretion of proteins into the host cell cytoplasm and for the insertion of chlamydial proteins (like the Inc set) into the inclusion membrane that separates the growing chlamydial microcolony from the host cell cytoplasm (see Montigiani et al (2002) Infection and Immunity 70(1); 386-379).

Proteins may be expressed and secreted by 2 hours (early cycle) after infection while the expression of other early and mid cycle Type III specific genes are not detectable until 6-12 hours (mid cycle). After 16-20 hours, the RBs begin to differentiate into EBs, and by 48-72 hours, the EBs predominate within the inclusion. Host cell lysis results in the release of the EBs to the extracellular space where they can infect more cells. For purposes of this description, an early gene is one that is expressed (in terms of mRNA expression) early in infection, an intermediate gene is one that is expressed in the mid-cycle after infection and a late gene is one which is expressed during the terminal transition of RBs to EBs. There may be a time lag between surface expression of early, mid and late stage proteins and their transcriptional and translational profiles because mRNA abundance may not always correlate with protein abundance.

In one example, the present invention may comprise TTSS effector proteins. The TTSS effector proteins as described are associated with the RB form of *Chlamydia pneumoniae* and may be identified, for example, using immunofluorescence microscopy (see Bannantine et al, Infection and Immunity 66(12); 6017-6021). Effector antibodies to putative Chlamydial TTSS effector proteins secreted by the TTSS machinery may be micro-injected into host cells at specified time points during *Chlamydia pneumoniae* infection (e.g., early, mid or late cycle). Host cell reaction to *Chlamydia pneumoniae* (e.g., actin remodeling, inhibition of endosomal maturation, host lipid acquisition, and MHC Class I and Class II molecule downregulation) associated with *Chlamydia pneumoniae* entry into host cells is then observed. Based on these temporal observations, TTSS effector proteins (RB-associated *Chlamydia pneumoniae* proteins) may be detected.

A specific composition of the present invention may comprise a combination of *Chlamydia pneumoniae* antigens, said combination consisting of two, three, four, five or all six *Chlamydia pneumoniae* antigens of a first antigen group, said first antigen group consisting of: (1) pmp2; (2) pmp10; (3) Enolase; (4) OmpH-like protein; and (5) the products of CPn specific genes CPn0759 and CPn0042. These antigens are referred to herein as the 'first antigen group'.

Preferably, the composition of the invention comprises a combination of *Chlamydia pneumoniae* antigens, said combination selected from the group consisting of: (1) pmp2 and pmp10; (2) pmp2 and Enolase; (3) pmp2 and OmpH-like protein; (4) pmp2 and CPn0759; (5) pmp2 and CPn0042; (6) pmp10 and Enolase; (7) pmp10 and

OmpH-like protein; (8) pmp10 and CPn0759; (9) pmp10 and CPn0042; (10) Enolase and OmpH-like protein (11) Enolase and CPn0759; (12) Enolase and CPn0042; (13) OmpH-like protein and CPn0759 (14) OmpH-like protein and CPn0042; (15) CPn0759 and CPn0042; (16) pmp2 and pmp10 and Enolase; (17) pmp2 and pmp10 and OmpH-like protein; (18) pmp2 and pmp10 and CPn0759; (19) pmp2 and pmp10 and CPn0042; (20) pmp2 and Enolase and OmpH-like protein; (21) pmp2 and Enolase and Cpn0759; (22) pmp2 and Enolase and CPn0042; (23) pmp2 and OmpH-like protein and CPn0759; (24) pmp2 and OmpH-like protein and CPn0042; (25) pmp2 and Cpn0759 and CPn0042; and (26) pmp10 and Enolase and OmpH-like protein; (27) pmp10 and Enolase and CPn0759; (28) pmp10 and Enolase and CPn0042; (29) Enolase and OmpH-like protein and CPn0759; (30) Enolase and OmpH-like protein and CPn0042; (31) OmpH-like protein and CPn0759 and CPn0042.

Preferably, the composition of *Chlamydia pneumoniae* antigens consists of pmp2, pmp10, Enolase, OmpH-like protein and CPn0759.

Preferably, the composition of *Chlamydia pneumoniae* antigens consists of pmp2, pmp10, Enolase, OmpH-like protein and CPn0042.

Preferably, the composition of *Chlamydia pneumoniae* antigens consists of pmp2, pmp10, Enolase, OmpH-like protein and CPn0759 and CPn0042.

The invention also provides for a slightly larger group of 12 *Chlamydia pneumoniae* antigens that are particularly suitable for immunisation purposes, particularly when used in combinations. (This second antigen group includes the six *Chlamydia pneumoniae* antigens of the first antigen group). These 12 *Chlamydia pneumoniae* antigens form a second antigen group of (1) pmp2; (2) pmp10; (3) Enolase; (4) OmpH-like protein; (5) CPn0759; (6) CPn0042; (7) ArtJ; (8) HtrA; (9) AtoS; (10) OmcA; (11) CPn0498; and (12) CPn0525. These antigens are referred to herein as the 'second antigen group'.

The invention therefore provides a composition comprising a combination of *Chlamydia pneumoniae* antigens, said combination selected from the group consisting of two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve *Chlamydia pneumoniae* antigens of the second antigen group. Preferably, the combination is selected from the group consisting of two, three, four or five *Chlamydia pneumoniae* antigens of the second antigen group. Still more preferably, the combination consists of six *Chlamydia pneumoniae* antigens of the second antigen group. Each of the *Chlamydia pneumoniae* antigens of the first and second antigen group are described in more detail below.

(1) Pmp10 (CPn0449)

One example of a pmp10 protein is set forth as SEQ ID NO: 1 below (GenBank Accession No.GI:14195016). Preferred pmp10 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 1; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 1, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These pmp2 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs,

mutants, *etc.*) of SEQ ID NO: 1. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 1. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 1. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 1

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   1 MKSQFSWLVL SSTLACFTSC STVFAATAEN IGPSDSFDGS TNTGTYTPKN TTTGIDYTLT
  61 GDITLQNLGD SAALTKGCFS DTTESLSFAG KGYSLSFLNI KSSAEGAALS VTTDKNLSLT
 121 GFSSLTFLAA PSSVITTPSG KGAVKCGGDL TFDNNGTILF KQDYCEENGG AISTKNLSLK
 181 NSTGSISFEG NKSSATGKKG GAICATGTVD ITNNTAPTLF SNNIAEAAGG AINSTGNCTI
 241 TGNTSLVFSE NSVTATAGNG GALSGDADVT ISGNQSVTFS GNQAVANGGA IYAKKLTLAS
 301 GGGGGISFSN NIVQGTTAGN GGAISILAAG ECSLSAEAGD ITFNGNAIVA TTPQTTKRNS
 361 IDIGSTAKIT NLRAISGHSI FFYDPITANT AADSTDTLNL NKADAGNSTD YSGSIVFSGE
 421 KLSEDEAKVA DNLTSTLKQP VTLTAGNLVL KRGVTLDTKG FTQTAGSSVI MDAGTTLKAS
 481 TEEVTLTGLS IPVDSLGEGK KVVIAASAAS KNVALSGPIL LLDNQGNAYE NHDLGKTQDF
 541 SFVQLSALGT ATTTDVPAVP TVATPTHYGY QGTWGMTWVD DTASTPKTKT ATLAWTNTGY
 601 LPNPERQGPL VPNSLWGSFS DIQAIQGVIE RSALTLCSDR GFWAAGVANF LDKDKKGEKR
 661 KYRHKSGGYA IGGAAQTCSE NLISFAFCQL FGSDKDFLVA KNHTDTYAGA FYIQHITECS
 721 GFIGCLLDKL PGSWSHKPLV LEGQLAYSHV SNDLKTKYTA YPEVKGSWGN NAFNMMLGAS
 781 SHSYPEYLHC FDTYAPYIKL NLTYIRQDSF SEKGTEGRSF DDSNLFNLSL PIGVKFEKFS
 841 DCNDFSYDLT LSYVPDLIRN DPKCTTALVI SGASWETYAN NLARQALQVR AGSHYAFSPM
 901 FEVLGQFVFE VRGSSRIYNV DLGGKFQF
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(2) Pmp2 = Polymorphic Outer Membrane Protein G Family (CPn 0013)

One example of a pmp2 protein is disclosed as SEQ ID NO[s]: 139 and 140 in WO 02/02606. {GenBank accession number: gi|4376270|gb|AAD18172.1 'CPn0013'; SEQ ID NO: 2 below}. Preferred pmp2 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 2; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 1, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These pmp2 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 2. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 1. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 2. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 2

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   1 MKIPLRFLLI SLVPTLSMSN LLGAATTEEL SASNSFDGTT STTSFSSKTS
  51 SATDGTNYVF KDSVVIENVP KTGETQSTSC FKNDAAAGDL NFLGGGFSFT
 101 FSNIDATTAS GAAIGSEAAN KTVTLSGFSA LSFLKSPAST VTNGLGAINV
 151 KGNLSLLDND KVLIQDNFST GDGGAINCAG SLKIANNKSL SFIGNSSSTR
 201 GGAIHTKNLT LSSGGETLFQ GNTAPTAAGK GGAIAIADSG TLSISGDSGD
 251 IIFEGNTIGA TGTVSHSAID LGTSAKITAL RAAQGHTIYF YDPITVTGST
 301 SVADALNINS PDTGDNKEYT GTIVFSGEKL TEAEAKDEKN RTSKLLQNVA
 351 FKNGTVVLKG DVVLSANGFS QDANSKLIMD LGTSLVANTE SIELTNLEIN
 401 IDSLRNGKKI KLSAATAQKD IRIDRPVVLA ISDESFYQNG FLNEDHSYDG
 451 ILELDAGKDI VISADSRSID AVQSPYGYQG KWTINWSTDD KKATVSWAKQ
 501 SFNPTAEQEA PLVPNLLWGS FIDVRSFQNF IELGTEGAPY EKRFWVAGIS
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 551  NVLHRSGREN QRKFRHVSGG AVVGASTRMP GGDTLSLGFA QLFARDKDYF
 601  MNTNFAKTYA GSLRLQHDAS LYSVVSILLG EGGLREILLP YVSKTLPCSF
 651  YGQLSYGHTD HRMKTESLPP PPPTLSTDHT SWGGYVWAGE LGTRVAVENT
 701  SGRGFFQEYT PFVKVQAVYA RQDSFVELGA ISRDFSDSHL YNLAIPLGIK
 751  LEKRFAEQYY HVVAMYSPDV CRSNPKCTTT LLSNQGSWKT KGSNLARQAG
 801  IVQASGFRSL GAAAELFGNF GFEWRGSSRS YNVDAGSKIK F*
```

(3) Enolase (Cpn0800)

One example of an 'Eno' protein is disclosed as SEQ ID NO[s]: 93 and 94 in WO 02/02606. {GenBank accession number: gi|4377111|gb|AAD18938.1|' Cpn0800'; SEQ ID NO: 3 below}.Preferred Eno proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 2; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 2, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Eno proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 3. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 3. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 3. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 3

```
   1  MFEAVIADIQ AREILDSRGY PTLHVKVTTS TGSVGEARVP SGASTGKKEA
  51  LEFRDTDSPR YQGKGVLQAV KNVKEILFPL VKGCSVYEQS LIDSLMMDSD
 101  GSPNKETLGA NAILGVSLAT AHAAAATLRR PLYRYLGGCF ACSLPCPMMN
 151  LINGGMHADN GLEFQEFMIR PIGASSIKEA VNMGADVFHT LKKLLHERGL
 201  STGVGDEGGF APNLASNEEA LELLLLAIEK AGFTPGKDIS LALDCAASSF
 251  YNVKTGTYDG RHYEEQIAIL SNLCDRYPID SIEDGLAEED YDGWALLTEV
 301  LGEKVQIVGD DLFVTNPELI LEGISNGLAN SVLIKPNQIG TLTETVYAIK
 351  LAQMAGYTTI ISHRSGETTD TTIADLAVAF NAGQIKTGSL SRSERVAKYN
 401  RLMEIEEELG SEAIFTDSNV FSYEDSEE*
```

(4) OmpH-like outer membrane protein (CPn0301)

One example of 'OmpH-like' protein is disclosed as SEQ ID NO[s]: 77 & 78 in WO 02/02606. {GenBank accession number: gi|4376577|gb|AAD18450.1| 'CPn0301'; SEQ ID NO: 4 below}. Preferred OmpH-like proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 4; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 3, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These OmpH-like proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 4. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 4. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more; preferably 19 or more, to remove the signal peptide) from the N-terminus of SEQ ID NO: 4. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 4

```
  1  MKKLLFSTFL LVLGSTSAAH ANLGYVNLKR CLEESDLGKK ETEELEAMKQ
 51  QFVKNAEKIE EELTSIYNKL QDEDYMESLS DSASEELRKK FEDLSGEYNA
101  YQSQYYQSIN QSNVKRIQKL IQEVKIAAES VRSKEKLEAI LNEEAVLAIA
151  PGTDKTTEII AILNESFKKQ N*
```

(5) CPn0042 (Hypothetical)

One example of hypothetical protein is set forth as SEQ ID NO: 5 below. GenBank accession number: gi|4376296|gb|AAD18195.1. Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 5; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 5, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 5. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 5. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more; preferably 19 or more, to remove the signal peptide) from the N-terminus of SEQ ID NO: 5 Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 5

```
  1 MEEVSEYLQQ VENQLESCSK RLTKMETFAL GVRLEAKEEI ESIILSDVVN RFEVLCRDIE
 61 DMLSRVEEIE RMLRMAELPL LPIKEALTKA FVQHNSCKEK LTKVEPYFKE SPAYLTSEER
121 LQSLNQTLQR AYKESQKVSG LESEVRACRE QLKDQVRQFE TQGVSLIKEE ILFVTSTFRT
181 KFSYHSFRLH VPCMRLYEEY YDDIDLERTR ARWMAMSERY RDAFQAFQEM LKEGLVEEAQ
241 ALRETEYWLY REERKSKKKH
```

(6) CPn0795 (Hypothetical)

One example of hypothetical protein is disclosed as SEQ ID NO[s]: 63 & 64 in WO 02/02606. {GenBank accession number: gi|4377106|gb|AAD18933.1| 'CPn0795'; SEQ ID NO: 6 below). As the examples demonstrate, we have shown for the first time that CPn0795 and related proteins in the group Cpn0794 – Cpn0799 have a secreted autotransporter function. It has been shown that proteins secreted by the autotransporter secretion mechanism possess an overall unifying structure, including an amino-terminal leader peptide (for secretion across the inner membrane), the secreted mature protein (or passenger domain), and a dedicated C-terminal domain, which forms a pore in the outer membrane through which the passenger domain passes to the cell surface. It is likely that requirements for secretion across the outer membrane are contained within a single molecule and secretion is an energy-independent process. Structural properties of the proteins may be confined by the size of the pore considering the biophysical constraints that may be imposed on secretion.

The autotransporter, or type V, secretion system is a dedicated protein translocation mechanism which allows the organism to secrete proteins to and beyond the bacterial surface. The secretion mechanism and the ability to develop a new autotransporter protein simply by a single recominbation event have presented bacteria with abundant

opportunities to increase the efficiency of secretion of proteins that were developed as periplasmic or exported virulence factors.

In one model of autotransporter (type V) secretion mechanism, proteins are exported by the autotransporter secretion mechanism and are translated as a polyproptein possessing domains. The autotransporters possess an overall unifying structure comprising three functional domains: the amino-termianl leader sequence, the secreted mature proterin (passenger domain) and a carboxy-terminal (beta-) domain that forms a beta-barrel pore to allow secretion of the passenger protein. The leader sequence directs secretion via the sec apparatus and is cleaved at the inner membrane by a signal peptidase releasing the remaining portion of the molecule into the periplasm. Once in the periplasm the β-domain assumes a biophysically favored state characterized by a β-barrel shaped structure which inserts itself into the outer membrane to form a pore. After insertion into the outer membrane the passenger domain is translocated to the bacterial cell surface where it may remain intact or undergo processing. A processed protein may be released into the extracellular milieu or remain associated with the bacterial cell surface. (Henderson and Nataro, "Virulence Functions of Autotransporter Proteins", Infection and Immunity, Vol. 69, No. 3, March 2001, pages 1231-1243).

Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 6; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 6, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 6. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 6. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more; preferably 19 or more, to remove the signal peptide) from the N-terminus of SEQ ID NO: 6. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). As the Examples demonstrate, we have shown for the first time that CPn0795 appears to be present and accessible to antibodies on the surface of the infectious EB form which makes this protein a good component of an immunogenic composition or vaccine.

Table 1 of this application demonstrates that Cpn0795 (SEQ ID NO: 6) a Cpn specific hypothetical protein is a FACS positive protein which demonstrates significant immunoprotective activity in a hamster spleen model of Chlamydia pneumoniae infection. We have found evidence to demonstrate that other Cpn proteins in this group of Cpn specific hypothetical proteins have now been found to have a secreted autotransporter function. These proteins, which are absent from *Chlamydia trachomatis* include: gi/4377105 (Cpn0794), gi/4377106 (Cpn0795), gi/4377107 (Cpn0796), gi/4377108 (Cpn0797), gi/4377109 (CPn0798), gi/4377110 (Cpn0799).

SEQ ID No 6

```
   1  MKDLGTLGGT SSTAKTVSPD GKVIMGRSQI ADGSWHAFMC HTDFSSNNVL
  51  FDLDNTYKTL RENGRQLNSI FNLQNMMLQR ASDHEFTEFG RSNIALGAGL
 101  YVNALQNLPS NLAAQYFGIA YKIRPKYRLG VFLDHNFSSH VPNNFNVSHN
 151  RLWMGAFIGW QDSDALGSSV KVSFGYGKQK ATITREQLEN TEAGSGESHF
 201  EGVAAQIEGR YGKSLGGHVR VQPFLGLQFV HITRKEYTEN AVQFPVHYDP
 251  IDYSTGVVYL GIGSHIALVD SLHVGTRMGM EQNFAAHTDR FSGSIASIGN
 301  FVFEKLDVTH TRAFAEMRVN YELPYLQSLN LILRVNQQPL QGVMGFSSDL
 351  RYALGF*
```

(7) ArtJ arginine periplasmic-binding protein (CPn 0482)

One example of 'ArtJ' protein is disclosed as SEQ ID NO[s]: 73 & 74 in WO 02/02606. {GenBank accession number: gi|4376767|gb|AAD18622.1| 'CPn0482'; SEQ ID NO: 7 below}. Preferred ArtJ proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 7; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 7, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These ArtJ proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 7. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 7. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 7. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). The ArtJ protein may be bound to a small molecule like arginine or another amino acid.

SEQ ID No 7

```
   1  MIKQIGRFFR AFIFIMPLSL TSCESKIDRN RIWIVGTNAT YPPFEYVDAQ
  51  GEVVGFDIDL AKAISEKLGK QLEVREFAFD ALILNLKKHR IDAILAGMSI
 101  TPSRQKEIAL LPYYGDEVQE LMVVSKRSLE TPVLPLTQYS SVAVQTGTFQ
 151  EHYLLSQPGI CVRSFDSTLE VIMEVRYGKS PVAVLEPSVG RVVLKDFPNL
 201  VATRLELPPE CWVLGCGLGV AKDRPEEIQT IQQAITDLKS EGVIQSLTKK
      WQLSEVAYE*
```

(8) HtrA DO Serine Protease (CPn0979)

One example of an 'HrtA' protein is disclosed as SEQ ID NO[s]: 111 & 112 in WO 02/02606. {GenBank accession number: gi|4377306|gb|AAD19116.1| 'CPn0979'; SEQ ID NO: 8 below}. Preferred HrtA proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 8; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 8, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These HrtA proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 8. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 8. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more; preferably at least 16 to remove the signal peptide) from the N-terminus of SEQ ID NO: 8. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic

domain, of a transmembrane domain, or of an extracellular domain). In relation to SEQ ID NO: 8, distinct domains are residues: 1-16; 17-497; 128-289; 290-381; 394-485; and 394-497.

SEQ ID No 8

```
   1  MITKQLRSWL AVLVGSSLLA LPLSGQAVGK KESRVSELPQ DVLLKEISGG
  51  FSKVATKATP AVVYIESFPK SQAVTHPSPG RRGPYENPFD YFNDEFFNRF
 101  FGLPSQREKP QSKEAVRGTG FLVSPDGYIV TNNHVVEDTG KIHVTLHDGQ
 151  KYPATVIGLD PKTDLAVIKI KSQNLPYLSF GNSDHLKVGD WAIAIGNPFG
 201  LQATVTVGVI SAKGRNQLHI ADFEDFIQTD AAINPGNSGG PLLNIDGQVI
 251  GVNTAIVSGS GGYIGIGFAI PSLMANRIID QLIRDGQVTR GFLGVTLQPI
 301  DAELAACYKL EKVYGALVTD VVKGSPADKA GLKQEDVIIA YNGKEVDSLS
 351  MFRNAVSLMN PDTRIVLKVV REGKVIEIPV TVSQAPKEDG MSALQRVGIR
 401  VQNLTPETAK KLGIAPETKG ILIISVEPGS VAASSGIAPG QLILAVNRQK
 451  VSSIEDLNRT LKDSNNENIL LMVSQGDVIR FIALKPEE*
```

(9) AtoS two-component regulatory system sensor histidine kinase protein (CPn0584)

One example of 'AtoS' protein is disclosed as SEQ ID NO^s: 105 & 106 in WO 02/02606. {GenBank accession number: gi|4376878|gb|AAD18723.1| 'CPn0584'; SEQ ID NO: 9 below}. Preferred AtoS proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 9; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 9, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These AtoS proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 9. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 9. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 9. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 9

```
  1  MNVPDSKNLH PPAYELLEIK ARITQSYKEA SAILTAIPDG ILLLSETGHF
 51  LICNSQAREI LGIDENLEIL NRSFTDVLPD TCLGFSIQEA LESLKVPKTL
101  RLSLCKESKE KEVELFIRKN EISGYLFIQI RDRSDYKQLE NAIERYKNIA
151  ELGKMTATLA HEIRNPLSGI VGFASILKKE ISSPRHQRML SSIISGTRSL
201  NNLVSSMLEY TKSQPLNLKI INLQDFFSSL IPLLSVSFPN CKFVREGAQP
251  LFRSIDPDRM NSVVWNLVKN AVETGNSPIT LTLHTSGDIS VTNPGTIPSE
301  IMDKLFTPFF TTKREGNGLG LAEAQKIIRL HGGDIQLKTS DSAVSFFIII
351  PELLAALPKE RAAS*
```

(10) OmcA 9kDa-cysteine-rich lipoprotein(CPn0558)

One example of 'OmcA' protein is disclosed as SEQ ID NO^s: 9 & 10 in WO 02/02606. {GenBank accession number: gi|4376850|gb|AAD18698.1| 'CPn0558', 'OmcA', 'Omp3'; SEQ ID NO: 10 below}. Preferred OmcA proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 10; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 10, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These OmcA proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 10. Preferred fragments of (b) comprise an

epitope from SEQ ID NO: 10. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more; preferably 18 or more to remove the signal peptide) from the N-terminus of SEQ ID NO: 10. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). The protein may be lipidated (*e.g.* by a *N*-acyl diglyceride), and may thus have a N-terminal cysteine.

SEQ ID No 10

```
  1 MKKAVLIAAM FCGVVSLSSC CRIVDCCFED PCAPSSCNPC EVIRKKERSC
 51 GGNACGSYVP SCSNPCGSTE CNSQSPQVKG CTSPDGRCKQ *
```

(11) CPn0498 (Hypothetical)

One example of a hypothetical protein is set forth as SEQ ID NO: 11 below. (GenBank Accession No. GI:4376784; AAD18638.1). Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 11; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 11, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 11. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 11. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more; preferably 18 or more to remove the signal peptide) from the N-terminus of SEQ ID NO: 11. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). The protein may be lipidated (*e.g.* by a *N*-acyl diglyceride), and may thus have a N-terminal cysteine.

SEQ ID No 11

```
  1 MNRRKARWVV ALFAMTALIS VGCCPWSQAK SRCSIDKYIP VVNRLLEVCG LPEAENVEDL
 61 IESSSAWVLT PEERFSGELV SICQVKDEHA FYNDLSLLHM TQAVPSYSAT YDCAVVFGGP
121 LPALRQRLDF LVREWQRGVR FKKIVFLCGE RGRYQSIEEQ EHFFDSRYNP FPTEENWESG
181 NRVTPSSEEE IAKFVWMQML LPRAWRDSTS GVRVTFLLAK PEENRVVANR KDTLLLFRSY
241 QEAFPGRVLF VSSQPFIGLD ACRVGQFFKG ESYDLAGPGF AQGVLKYHWA PRICLHTLAE
301 WLKETNGCLN ISEGCFG
```

(12) CPn 0525 (hypothetical)

One example of 'Cpn0525' protein is disclosed as SEQ ID NO[s]: 117 & 118 in WO 02/02606. {GenBank accession number: gi|4376814|gb|AAD18665.1| 'CPn0525', SEQ ID NO: 12 below}. Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 12; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 12, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These OmcA proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs,

mutants, *etc.*) of SEQ ID NO: 12. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 12. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more; preferably 18 or more to remove the signal peptide) from the N-terminus of SEQ ID NO: 12. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 12

```
  1  MHDALLSILA IQELDIKMIR LMRVKKEHQK ELAKVQSLKS DIRRKVQEKE
 51  LEMENLKTQI RDGENRIQEI SEQINKLENQ QAAVKKMDEF NALTQEMTTA
101  NKERRSLEHQ LSDLMDKQAG GEDLIVSLKE SLASTENSSS VIEKEIFESI
151  KKINEEGKAL LEQRTELKHA TNPELLSIYE RLLNNKKDRV VVPIENRVCS
201  GCHIVLTPQH ENLVRKKDRL IFCEHCSRIL YWQESQVNAQ ENSTAKRRRR
251  RAAV*
```

Third Antigen Group

The immunogenicity of other *Chlamydia pneumoniae* antigens may be improved by combination with two or more *Chlamydia pneumoniae* antigens from either the first antigen group or the second antigen group. Such other *Chlamydia pneumoniae* antigens include a third antigen group consisting of (1) LcrE, (2) DnaK, (3) Omp85 homolog, (4) Mip-like; (5) OmcB (6) MurG (7) Cpn0186 and (8) fliY. These antigens are referred to herein as the "third antigen group".

(13) LcrE low calcium response E protein (CPn0324)

One example of a 'LcrE' protein is disclosed as SEQ ID NO[s]: 29 & 30 in WO 02/02606. {GenBank accession number: gi|4376602|gb|AAD18473.1| 'CPn0324'; SEQ ID NO: 13 below}. Preferred LcrE proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 13; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 13, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These LcrE proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 13. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 13. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 13. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 13

```
  1  MAASGGTGGL GGTQGVNLAA VEAAAAKADA AEVVASQEGS EMNMIQQSQD
 51  LTNPAAATRT KKKEEKFQTL ESRKKGEAGK AEKKSESTEE KPDTDLADKY
101  ASGNSEISGQ ELRGLRDAIG DDASPEDILA LVQEKIKDPA LQSTALDYLV
151  QTTPPSQGKL KEALIQARNT HTEQFGRTAI GAKNILFASQ EYADQLNVSP
201  SGLRSLYLEV TGDTHTCDQL LSMLQDRYTY QDMAIVSSFL MKGMATELKR
251  QGPYVPSAQL QVLMTETRNL QAVLTSYDYF ESRVPILLDS LKAEGIQTPS
301  DLNFVKVAES YHKIINDKFP TASKVEREVR NLIGDDVDSV TGVLNLFFSA
351  LRQTSSRLFS SADKRQQLGA MIANALDAVN INNEDYPKAS DFPKPYPWS*
```

(14) DnaK heat-shock protein 70 (chaperone) (CPn0503)

One example of 'DnaK' protein is disclosed as SEQ ID NO[s]: 103 & 104 in WO 02/02606. {GenBank accessionnumber: gi|4376790|gb|AAD18643.1| 'CPn0503'; SEQ ID NO: 14 below. Preferred DnaK proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 14; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 14, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These DnaK proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 14. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 14. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 14. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 14

```
  1  MSEHKKSSKI IGIDLGTTNS CVSVMEGGQA KVITSSEGTR TTPSIVAFKG
 51  NEKLVGIPAK RQAVTNPEKT LGSTKRFIGR KYSEVASEIQ TVPYTVTSGS
101  KGDAVFEVDG KQYTPEEIGA QILMKMKETA EAYLGETVTE AVITVPAYFN
151  DSQRASTKDA GRIAGLDVKR IIPEPTAAAL AYGIDKVGDK KIAVFDLGGG
201  TFDISILEIG DGVFEVLSTN GDTLLGGDDF DEVIIKWMIE EFKKQEGIDL
251  SKDNMALQRL KDAAEKAKIE LSGVSSTEIN QPFITMDAQG PKHLALTLTR
301  AQFEKLAASL IERTKSPCIK ALSDAKLSAK DIDDVLLVGG MSRMPAVQET
351  VKELFGKEPN KGVNPDEVVA IGAAIQGGVL GGEVKDVLLL DVIPLSLGIE
401  TLGGVMTTLV ERNTTIPTQK KQIFSTAADN QPAVTIVVLQ GERPMAKDNK
451  EIGRFDLTDI PPAPRGHPQI EVSFDIDANG IFHVSAKDVA SGKEQKIRIE
501  ASSGLQEDEI QRMVRDAEIN KEEDKKRREA SDAKNEADSM IFRAEKAIKD
551  YKEQIPETLV KEIEERIENV RNALKDDAPI EKIKEVTEDL SKHMQKIGES
601  MQSQSASAAA SSAANAKGGP NINTEDLKKH SFSTKPPSNN GSSEDHIEEA
651  DVEIIDNDDK*
```

(15) Omp85 homolog (Cpn0300)

One example of an Omp85 Homolog protein is disclosed as SEQ ID NO[s]: 147 & 148 in WO 02/02606. {GenBank accession number: gi|4376576|gb|AAD18449.1| 'CPn0300'; SEQ ID NO: 15 below}. Preferred Omp85 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 15; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 15, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These DnaK proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 15. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 15. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 15. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 15

```
   1  MLIMRNKVIL QISILALIQT PLTLFSTEKV KEGHVVVDSI TIITEGENAS
  51  NKHPLPKLKT RSGALFSQLD FDEDLRILAK EYDSVEPKVE FSEGKTNIAL
 101  HLIAKPSIRN IHISGNQVVP EHKILKTLQI YRNDLFEREK FLKGLDDLRT
 151  YYLKRGYFAS SVDYSLEHNQ EKGHIDVLIK INEGPCGKIK QLTFSGISRS
 201  EKSDIQEFIQ TKQHSTTTSW FTGAGLYHPD IVEQDSLAIT NYLHNNGYAD
 251  AIVNSHYDLD DKGNILLYMD IDRGSRYTLG HVHIQGFEVL PKRLIEKQSQ
 301  VGPNDLYCPD KIWDGAHKIK QTYAKYGYIN TNVDVLFIPH ATRPIYDVTY
 351  EVSEGSPYKV GLIKITGNTH TKSDVILHET SLFPGDTFNR LKLEDTEQRL
 401  RNTGYFQSVS VYTVRSQLDP MGNADQYRDI FVEVKETTTG NLGLFLGFSS
 451  LDNLFGGIEL SESNFDLFGA RNIFSKGFRC LRGGGEHLFL KANFGDKVTD
 501  YTLKWTKPHF LNTPWILGIE LDKSINRALS KDYAVQTYGG NVSTTYILNE
 551  HLKYGLFYRG SQTSLHEKRK FLLGPNIDSN KGFVSAAGVN LNYDSVDSPR
 601  TPTTGIRGGV TFEVSGLGGT YHFTKLSLNS SIYRKLTRKG ILKIKGEAQF
 651  IKPYSNTTAE GVPVSERFFL GGETTVRGYK SFIIGPKYSA TEPQGGLSSL
 701  LISEEFQYPL IRQPNISAFV FLDSGFVGLQ EYKISLKDLR SSAGFGLRFD
 751  VMNNVPVMLG FGWPFRPTET LNGEKIDVSQ RFFFALGGMF *
```

(16) Mip-like FKBP-type peptidyl-prolyl cis-trans (CPn0661)

One example of a Mip-like protein is disclosed as SEQ ID NO[s]: 55 & 56 in WO 02/02606. {GenBank accession number: gi|4376960|gb|AAD18800.1| 'CPn0661'; SEQ ID NO: 16 below}. Preferred Mip-like proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 16; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 16, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These mip-like proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 16. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 16. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 16. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 16

```
   1  MNRRWNLVLA TVALALSVAS CDVRSKDKDK DQGSLVEYKD NKDTNDIELS
  51  DNQKLSRTFG HLLARQLRKS EDMFFDIAEV AKGLQAELVC KSAPLTETEY
 101  EEKMAEVQKL VFEKKSKENL SLAEKFLKEN SKNAGVVEVQ PSKLQYKIIK
 151  EGAGKAISGK PSALLHYKGS FINGQVFSSS EGNNEPILLP LGQTIPGFAL
 201  GMQGMKEGET RVLYIHPDLA YGTAGQLPPN SLLIFEINLI QASADEVAAV
 251  PQEGNQGE*
```

(17) OmcB 60 kDa Cysteine rich OMP (CPn0557)

One example of an OmcB protein is disclosed as SEQ ID NO[s]: 47 & 48 in WO 02/02606. {GenBank accession number: gi|4376849|gb|AAD18697.1| 'CPn0557'; SEQ ID NO: 17 below}. Preferred OmcB proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 17; and/or (b) which is a fragment of at least *n*

consecutive amino acids of SEQ ID NO: 17, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These OmcB proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 17. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 17. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 17. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain)..

SEQ ID No 17

```
   1  MSKLIRRVVT VLALTSMASC FASGGIEAAV AESLITKIVA SAETKPAPVP
  51  MTAKKVRLVR RNKQPVEQKS RGAFCDKEFY PCEEGRCQPV EAQQESCYGR
 101  LYSVKVNDDC NVEICQSVPE YATVGSPYPI EILAIGKKDC VDVVITQQLP
 151  CEAEFVSSDP ETTPTSDGKL VWKIDRLGAG DKCKITVWVK PLKEGCCFTA
 201  ATVCACPELR SYTKCGQPAI CIKQEGPDCA CLRCPVCYKI EVVNTGSAIA
 251  RNVTVDNPVP DGYSHASGQR VLSFNLGDMR PGDKKVFTVE FCPQRRGQIT
 301  NVATVTYCGG HKCSANVTTV VNEPCVQVNI SGADWSYVCK PVEYSISVSN
 351  PGDLVLHDVV IQDTLPSGVT VLEAPGGEIC CNKVVWRIKE MCPGETLQFK
 401  LVVKAQVPGR FTNQVAVTSE SNCGTCTSCA ETTTHWKGLA ATHMCVLDTN
 451  DPICVGENTV YRICVTNRGS AEDTNVSLIL KFSKELQPIA SSGPTKGTIS
 501  GNTVVFDALP KLGSKESVEF SVTLKGIAPG DARGEAILSS DTLTSPVSDT
 551  ENTHVY*
```

(18) MurG peptidoglycan transferase protein (CPn0904)

One example of a 'MurG' protein is disclosed as SEQ ID NO[s]: 107 & 108 in WO 02/02606. {GenBank accession number: gi|4377224|gb|AAD19042.1| 'CPn0904'; SEQ ID NO: 18 below}. Preferred MurG proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 18; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 18, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These MurG proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 18. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 18. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 18. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). The MurG may be lipidated *e.g.* with undecaprenyl.

SEQ ID No 18

```
   1  MMKKIRKVAL AVGGSGGHIV PALSVKEAFS REGIDVLLLG KGLKNHPSLQ
  51  QGISYREIPS GLPTVLNPIK IMSRTLSLCS GYLKARKELK IFDPDLVIGF
 101  GSYHSLPVLL AGLSHKIPLF LHEQNLVPGK VNQLFSRYAR GIGVNFSPVT
 151  KHFRCPAEEV FLPKRSFSLG SPMMKRCTNH TPTICVVGGS QGAQILNTCV
 201  PQALVKLVNK YPNMYVHHIV GPKSDVMKVQ HVYNRGEVLC CVKPFEEQLL
 251  DVLLAADLVI SRAGATILEE ILWAKVPGIL IPYPGAYGHQ EVNAKFFVDV
 301  LEGGTMILEK ELTEKLLVEK VTFALDSHNR EKQRNSLAAY SQQRSTKTFH
 351  AFICECL*
```

(19) CPn0186 (Hypothetical)

One example of a hypothetical protein is set forth as SEQ ID NO: 19 below}. (GenBank Accession No. GI:4376456; AAD18339.1). Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 19; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 19, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 19. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 19. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 19. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 19

```
  1 MSSPVNNTPS APNIPIPAPT TPGIPTTKPR SSFIEKVIIV AKYILFAIAA TSGALGTILG
 61 LSGALTPGIG IALLVIFFVS MVLLGLILKD SISGGEERRL REEVSRFTSE NQRLTVITTT
121 LETEVKDLKA AKDQLTLEIE AFRNENGNLK TTAEDLEEQV SKLSEQLEAL ERINQLIQAN
181 AGDAQEISSE LKKLISGWDS KVVEQINTSI QALKVLLGQE WVQEAQTHVK AMQEQIQALQ
241 AEILGMHNQS TALQKSVENL LVQDQALTRV VGELLESENK LSQACSALRQ EIEKLAQHET
301 SLQQRIDAML AQEQNLAEQV TALEKMKQEA QKAESEFIAC VRDRTFGRRE TPPPTTPVVE
361 GDESQEEDEG GTPPVSQPSS PVDRATGDGQ
```

(20) FliY Glutamine Binding Protein (CPn0604)

One example of a hypothetical protein is set forth as SEQ ID NO[s]: 11 & 12 in WO 02/02606. {GenBank accession number: gi|4376900|gb|AAD18743.1| 'CPn0604'; SEQ ID NO: 20 below}. Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 20; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 20, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 20. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 20. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 20. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 20

```
  1 MKIKFSWKVN FLICLLAVGL IFFGCSRVKR EVLVGRDATW FPKQFGIYTS
 51 DTNAFLNDLV SEINYKENLN INIVNQDWVH LFENLDDKKT QGAFTSVLPT
101 LEMLEHYQFS DPILLTGPVL VVAQDSPYQS IEDLKGRLIG VYKFDSSVLV
151 AQNIPDAVIS LYQHVPIALE ALTSNCYDAL LAPVIEVTAL IETAYKGRLK
201 IISKPLNADG LRLAILKGTN GDLLEGFNAG LVKTRRSGKY DAIKQRYRLP
```

The immunogenicity of other *Chlamydia pneumoniae* antigens may be improved by combination with two or more *Chlamydia pneumoniae* antigens from either the first antigen group or the second antigen group or the third antigen group. Such other *Chlamydia pneumoniae* antigens include a fourth antigen group consisting one or more members of the PMP family. These antigens are referred to herein as the "fourth antigen group". Each of the *Chlamydia pneumoniae* antigens of the fourth antigen group is described in more detail below.

Fourth Antigen Group

(21) Polymorphic Membrane Proteins (PMP)

A family of **twenty one** *Chlamydia pneumoniae* genes encoding predicted polymorphic membrane proteins (PMP) have been identified (*pmp1* to *pmp21*).

Pmp1 (CPn0005)

One example of a Pmp1 protein is set forth as SEQ ID NO[s]: 41 & 42 in WO 02/02606. {GenBank accession number: gi|4376260|gb|AAD18163.1 'CPn0005'; SEQ ID NO: 21 below}.

SEQ ID No 21

```
   1  MRFSLCGFPL VFSFTLLSVF DTSLSATTIS LTPEDSFHGD SQNAERSYNV
  51  QAGDVYSLTG DVSISNVDNS ALNKACFNVT SGSVTFAGNH HGLYFNNISS
 101  GTTKEGAVLC CQDPQATARF SGFSTLSFIQ SPGDIKEQGC LYSKNALMLL
 151  NNYVVRFEQN QSKTKGGAIS GANVTIVGNY DSVSFYQNAA TFGGAIHSSG
 201  PLQIAVNQAE IRFAQNTAKN GSGGALYSDG DIDIDQNAYV LFRENEALTT
 251  AIGKGGAVCC LPTSGSSTPV PIVTFSDNKQ LVFERNHSIM GGGAIYARKL
 301  SISSGGPTLF INNISYANSQ NLGGAIAIDT GGEISLSAEK GTITFQGNRT
 351  SLPFLNGIHL LQNAKFLKLQ ARNGYSIEFY DPITSEADGS TQLNINGDPK
 401  NKEYTGTILF SGEKSLANDP RDFKSTIPQN VNLSAGYLVI KEGAEVTVSK
 451  FTQSPGSHLV LDLGTKLIAS KEDIAITGLA IDIDSLSSSS TAAVIKANTA
 501  NKQISVTDSI ELISPTGNAY EDLRMRNSQT FPLLSLEPGA GGSVTVTAGD
 551  FLPVSPHYGF QGNWKLAWTG TGNKVGEFFW DKINYKPRPE KEGNLVPNIL
 601  WGNAVDVRSL MQVQETHASS LQTDRGLWID GIGNFFHVSA SEDNIRYRHN
 651  SGGYVLSVNN EITPKHYTSM AFSQLFSRDK DYAVSNNEYR MYLGSYLYQY
 701  TTSLGNIFRY ASRNPNVNVG ILSRRFLQNP LMIFHFLCAY GHATNDMKTD
 751  YANFPMVKNS WRNNCWAIEC GGSMPLLVFE NGRLFQGAIP FMKLQLVYAY
 801  QGDFKETTAD GRRFSNGSLT SISVPLGIRF EKLALSQDVL YDFSFSYIPD
 851  IFRKDPSCEA ALVISGDSWL VPAAHVSRHA FVGSGTGRYH FNDYTELLCR
 901  GSIECRPHAR NYNINCGSKF RF*
```

Pmp 4 (CPn0017)

One example of a Pmp 4 protein is designated SEQ ID NO: 22. The sequence for pmp4 protein can be found at AE001587.1 GI:4376271.

Pmp 6 (CPn0444)

One example of a Pmp 6 protein is set forth as SEQ ID NO[s] 31 & 32 in WO 02/02606. {GenBank accession number: gi|4376727|gb|AAD18588.1| 'CPn0444'; SEQ ID NO: 23 below}.

SEQ ID No 23

```
   1  MKYSLPWLLT SSALVFSLHP LMAANTDLSS SDNYENGSSG SAAFTAKETS
  51  DASGTTYTLT SDVSITNVSA ITPADKSCFT NTGGALSFVG ADHSLVLQTI
```

```
 101  ALTHDGAAIN NTNTALSFSG FSSLLIDSAP ATGTSGGKGA ICVTNTEGGT
 151  ATFTDNASVT LQKNTSEKDG AAVSAYSIDL AKTTTAALLD QNTSTKNGGA
 201  LCSTANTTVQ GNSGTVTFSS NTATDKGGGI YSKEKDSTLD ANTGVVTFKS
 251  NTAKTGGAWS SDDNLALTGN TQVLFQENKT TGSAAQANNP EGCGGAICCY
 301  LATATDKTGL AISQNQEMSF TSNTTTANGG AIYATKCTLD GNTTLTFDQN
 351  TATAGCGGAI YTETEDFSLK GSTGTVTFST NTAKTGGALY SKGNSSLTGN
 401  TNLLFSGNKA TGPSNSSANQ EGCGGAILAF IDSGSVSDKT GLSIANNQEV
 451  SLTSNAATVS GGAIYATKCT LTGNGSLTFD GNTAGTSGGA IYTETEDFTL
 501  TGSTGTVTFS TNTAKTGGAL YSKGNNSLSG NTNLLFSGNK ATGPSNSSAN
 551  QEGCGGAILS FLESASVSTK KGLWIEDNEN VSLSGNTATV SGGAIYATKC
 601  ALHGNTTLTF DGNTAETAGG AIYTETEDFT LTGSTGTVTF STNTAKTAGA
 651  LHTKGNTSFT KNKALVFSGN SATATATTTT DQEGCGGAIL CNISESDIAT
 701  KSLTLTENES LSFINNTAKR SGGGIYAPKC VISGSESINF DGNTAETSGG
 751  AIYSKNLSIT ANGPVSFTNN SGGKGGAIYI ADSGELSLEA IDGDITFSGN
 801  RATEGTSTPN SIHLGAGAKI TKLAAAPGHT IYFYDPITME APASGGTIEE
 851  LVINPVVKAI VPPPQPKNGP IASVPVVPVA PANPNTGTIV FSSGKLPSQD
 901  ASIPANTTTI LNQKINLAGG NVVLKEGATL QVYSFTQQPD STVFMDAGTT
 951  LETTTTNNTD GSIDLKNLSV NLDALDGKRM ITIAVNSTSG GLKISGDLKF
1001  HNNEGSFYDN PGLKANLNLP FLDLSSTSGT VNLDDFNPIP SSMAAPDYGY
1051  QGSWTLVPKV GAGGKVTLVA EWQALGYTPK PELRATLVPN SLWNAYVNIH
1101  SIQQEIATAM SDAPSHPGIW IGGIGNAFHQ DKQKENAGFR LISRGYIVGG
1151  SMTTPQEYTF AVAFSQLFGK SKDYVVSDIK SQVYAGSLCA QSSYVIPLHS
1201  SLRRHVLSKV LPELPGETPL VLHGQVSYGR NHHNMTTKLA NNTQGKSDWD
1251  SHSFAVEVGG SLPVDLNYRY LTSYSPYVKL QVVSVNQKGF QEVAADPRIF
1301  DASHLVNVSI PMGLTFKHES AKPPSALLLT LGYAVDAYRD HPHCLTSLTN
1351  GTSWSTFATN LSRQAFFAEA SGHLKLLHGL DCFASGSCEL RSSSRSYNAN
1401  CGTRYSF*
```

Pmp 7 (CPn0445)

One example of a Pmp 7 protein is set forth as SEQ ID NO[s] 153 & 154 in WO 02/02606. {GenBank accession number: gi|4376728|gb|AAD18589.1| 'CPn0445'; SEQ ID NO: 24 below}.

SEQ ID No 24

```
   1  MKSSVSWLFF SSIPLFSSLS IVAAEVTLDS SNNSYDGSNG TTFTVFSTTD
  51  AAAGTTYSLL SDVSFQNAGA LGIPLASGCF LEAGGDLTFQ GNQHALKFAF
 101  INAGSSAGTV ASTSAADKNL LFNDFSRLSI ISCPSLLLSP TGQCALKSVG
 151  NLSLTGNSQI IFTQNFSSDN GGVINTKNFL LSGTSQFASF SRNQAFTGKQ
 201  GGVVYATGTI TIENSPGIVS FSQNLAKGSG GALYSTDNCS ITDNFQVIFD
 251  GNSAWEAAQA QGGAICCTTT DKTVTLTGNK NLSFTNNTAL TYGGAISGLK
 301  VSISAGGPTL FQSNISGSSA GQGGGGAINI ASAGELALSA TSGDITFNNN
 351  QVTNGSTSTR NAINIIDTAK VTSIRAATGQ SIYFYDPITN PGTAASTDTL
 401  NLNLADANSE IEYGGAIVFS GEKLSPTEKA IAANVTSTIR QPAVLARGDL
 451  VLRDGVTVTF KDLTQSPGSR ILMDGGTTLS AKEANLSLNG LAVNLSSLDG
 501  TNKAALKTEA ADKNISLSGT IALIDTEGSF YENHNLKSAS TYPLLELTTA
 551  GANGTITLGA LSTLTLQEPE THYGYQGNWQ LSWANATSSK IGSINWTRTG
 601  YIPSPERKSN LPLNSLWGNF IDIRSINQLI ETKSSGEPFE RELWLSGIAN
 651  FFYRDSMPTR HGFRHISGGY ALGITATTPA EDQLTFAFCQ LFARDRNHIT
 701  GKNHGDTYGA SLYFHHTEGL FDIANFLWGK ATRAPWVLSE ISQIIPLSFD
 751  AKFSYLHTDN HMKTYYTDNS IIKGSWRNDA FCADLGASLP FVISVPYLLK
 801  EVEPFVKVQY IYAHQQDFYE RHAEGRAFNK SELINVEIPI GVTFERDSKS
 851  EKGTYDLTLM YILDAYRRNP KCQTSLIASD ANWMAYGTNL ARQGFSVRAA
 901  NHFQVNPHME IFGQFAFEVR SSSRNYNTNL GSKFCF*
```

Pmp 8 (CPn0446)

One example of a Pmp 8 protein is set forth as SEQ ID NO[s] 45 & 46 in WO 02/02606. {GenBank accession number: gi|4376729|gb|AAD18590.1| 'CPn0446'; SEQ ID NO: 25 below}.

SEQ ID No 25

```
   1  MKIPLHKLLI SSTLVTPILL SIATYGADAS LSPTDSFDGA GGSTFTPKST
  51  ADANGTNYVL SGNVYINDAG KGTALTGCCF TETTGDLTFT GKGYSFSFNT
 101  VDAGSNAGAA ASTTADKALT FTGFSNLSFI AAPGTTVASG KSTLSSAGAL
 151  NLTDNGTILF SQNVSNEANN NGGAITTKTL SISGNTSSIT FTSNSAKKLG
 201  GAIYSSAAAS ISGNTGQLVF MNNKGETGGG ALGFEASSSI TQNSSLFFSG
 251  NTATDAAGKG GAIYCEKTGE TPTLTISGNK SLTFAENSSV TQGGAICAHG
 301  LDLSAAGPTL FSNNRCGNTA AGKGGAIAIA DSGSLSLSAN QGDITFLGNT
 351  LTSTSAPTST RNAIYLGSSA KITNLRAAQG QSIYFYDPIA SNTTGASDVL
 401  TINQPDSNSP LDYSGTIVFS GEKLSADEAK AADNFTSILK QPLALASGTL
 451  ALKGNVELDV NGFTQTEGST LLMQPGTKLK ADTEAISLTK LVVDLSALEG
 501  NKSVSIETAG ANKTITLTSP LVFQDSSGNF YESHTINQAF TQPLVVFTAA
 551  TAASDIYIDA LLTSPVQTPE PHYGYQGHWE ATWADTSTAK SGTMTWVTTG
 601  YNPNPERRAS VVPDSLWASF TDIRTLQQIM TSQANSIYQQ RGLWASGTAN
 651  FFHKDKSGTN QAFRHKSYGY IVGGSAEDFS ENIFSVAFCQ LFGKDKDLFI
 701  VENTSHNYLA SLYLQHRAFL GGLPMPSFGS ITDMLKDIPL ILNAQLSYSY
 751  TKNDMDTRYT SYPEAQGSWT NNSGALELGG SLALYLPKEA PFFQGYFPFL
 801  KFQAVYSRQQ NFKESGAEAR AFDDGDLVNC SIPVGIRLEK ISEDEKNNFE
 851  ISLAYIGDVY RKNPRSRTSL MVSGASWTSL CKNLARQAFL ASAGSHLTLS
 901  PHVELSGEAA YELRGSAHIY NVDCGLRYSF *
```

Pmp 9 (CPn0447)

One example of a Pmp 9 protein is set forth as SEQ ID NO[s] 33 & 34 in WO 02/02606. {GenBank accession number: gi|4376731|gb|AAD18591.1| 'CPn0447'; SEQ ID NO: 26 below}.

SEQ ID No 26

```
   1  MKSSLHWFLI SSSLALPLSL NFSAFAAVVE INLGPTNSFS GPGTYTPPAQ
  51  TTNADGTIYN LTGDVSITNA GSPTALTASC FKETTGNLSF QGHGYQFLLQ
 101  NIDAGANCTF TNTAANKLLS FSGFSYLSLI QTTNATTGTG AIKSTGACSI
 151  QSNYSCYFGQ NFSNDNGGAL QGSSISLSLN PNLTFAKNKA TQKGGALYST
 201  GGITINNTLN SASFSENTAA NNGGAIYTEA SSFISSNKAI SFINNSVTAT
 251  SATGGAIYCS STSAPKPVLT LSDNGELNFI GNTAITSGGA IYTDNLVLSS
 301  GGPTLFKNNS AIDTAAPLGG AIAIADSGSL SLSALGGDIT FEGNTVVKGA
 351  SSSQTTTRNS INIGNTNAKI VQLRASQGNT IYFYDPITTS ITAALSDALN
 401  LNGPDLAGNP AYQGTIVFSG EKLSEAEAAE ADNLKSTIQQ PLTLAGGQLS
 451  LKSGVTLVAK SFSQSPGSTL LMDAGTTLET ADGITINNLV LNVDSLKETK
 501  KATLKATQAS QTVTLSGSLS LVDPSGNVYE DVSWNNPQVF SCLTLTADDP
 551  ANIHITDLAA DPLEKNPIHW GYQGNWALSW QEDTATKSKA ATLTWTKTGY
 601  NPNPERRGTL VANTLWGSFV DVRSIQQLVA TKVRQSQETR GIWCEGISNF
 651  FHKDSTKINK GFRHISAGYV VGATTTLASD NLITAAFCQL FGKDRDHFIN
 701  KNRASAYAAS LHLQHLATLS SPSLLRYLPG SESEQPVLFD AQISYIYSKN
 751  TMKTYYTQAP KGESSWYNDG CALELASSLP HTALSHEGLF HAYFPFIKVE
 801  ASYIHQDSFK ERNTTLVRSF DSGDLINVSV PIGITFERFS RNERASYEAT
 851  VIYVADVYRK NPDCTTALLI NNTSWKTTGT NLSRQAGIGR AGIFYAFSPN
 901  LEVTSNLSME IRGSSRSYNA DLGGKFQF*
```

Pmp 11 (CPn0451)

One example of a Pmp 11 protein is set forth as SEQ ID NO[s] 115 & 116 in WO 02/02606. {GenBank accession number: gi|4376733|gb|AAD18593.1| 'CPn0451'; SEQ ID NO: 27 below).

SEQ ID No 27

```
   1  MKTSIPWVLV SSVLAFSCHL QSLANEELLS PDDSFNGNID SGTFTPKTSA
  51  TTYSLTGDVF FYEPGKGTPL SDSCFKQTTD NLTFLGNGHS LTFGFIDAGT
 101  HAGAAASTTA NKNLTFSGFS LLSFDSSPST TVTTGQGTLS SAGGVNLENI
 151  RKLVVAGNFS TADGGAIKGA SFLLTGTSGD ALFSNNSSST KGGAIATTAG
 201  ARIANNTGYV RFLSNIASTS GGAIDDEGTS ILSNNKFLYF EGNAAKTTGG
 251  AICNTKASGS PELIISNNKT LIFASNVAET SGGAIHAKKL ALSSGGFTEF
```

```
301  LRNNVSSATP KGGAISIDAS GELSLSAETG NITFVRNTLT TTGSTDTPKR
351  NAINIGSNGK FTELRAAKNH TIFFYDPITS EGTSSDVLKI NNGSAGALNP
401  YQGTILFSGE TLTADELKVA DNLKSSFTQP VSLSGGKLLL QKGVTLESTS
451  FSQEAGSLLG MDSGTTLSTT AGSITITNLG INVDSLGLKQ PVSLTAKGAS
501  NKVIVSGKLN LIDIEGNIYE SHMFSHDQLF SLLKITVDAD VDTNVDISSL
551  IPVPAEDPNS EYGFQGQWNV NWTTDTATNT KEATATWTKT GFVPSPERKS
601  ALVCNTLWGV FTDIRSLQQL VEIGATGMEH KQGFWVSSMT NFLHKTGDEN
651  RKGFRHTSGG YVIGGSAHTP KDDLFTFAFC HLFARDKDCF IAHNNSRTYG
701  GTLFFKHSHT LQPQNYLRLG RAKFSESAIE KFPREIPLAL DVQVSFSHSD
751  NRMETHYTSL PESEGSWSNE CIAGGIGLDL PFVLSNPHPL FKTFIPQMKV
801  EMVYVSQNSF FESSSDGRGF SIGRLLNLSI PVGAKFVQGD IGDSYTYDLS
851  GFFVSDVYRN NPQSTATLVM SPDSWKIRGG NLSRQAFLLR GSNNYVYNSN
901  CELFGHYAME LRGSSRNYNV DVGTKLRF*
```

Pmp 12 (CPn0452)

One example of a Pmp 12 protein is set forth as SEQ ID NO[s] 51 & 52 in WO 02/02606. {GenBank accession number: gi|4376735|gb|AAD18594.1 'CPn0452'; SEQ ID NO: 28 below).

SEQ ID No 28

```
  1  MTILRNFLTC SALFLALPAA AQVVYLHESD GYNGAINNKS LEPKITCYPE
 51  GTSYIFLDDV RISNVKHDQE DAGVFINRSG NLFFMGNRCN FTFHNLMTEG
101  FGAAISNRVG DTTLTLSNFS YLAFTSAPLL PQGQGAIYSL GSVMIENSEE
151  VTFCGNYSSW SGAAIYTPYL LGSKASRPSV NLSGNRYLVF RDNVSQGYGG
201  AISTHNLTLT TRGPSCFENN HAYHDVNSNG GAIAIAPGGS ISISVKSGDL
251  IFKGNTASQD GNTIHNSIHL QSGAQFKNLR AVSESGVYFY DPISHSESHK
301  ITDLVINAPE GKETYEGTIS FSGLCLDDHE VCAENLTSTI LQDVTLAGGT
351  LSLSDGVTLQ LHSFKQEASS TLTMSPGTTL LCSGDARVQN LHILIEDTDN
401  FVPVRIRAED KDALVSLEKL KVAFEAYWSV YDFPQFKEAF TIPLLELLGP
451  SFDSLLLGET TLERTQVTTE NDAVRGFWSL SWEEYPPSLD KDRRITPTKK
501  TVFLTWNPEI TSTP*
```

Pmp 13 (CPn0453)

One example of a Pmp 13 protein is set forth as SEQ ID NO[s] 3 & 4 in WO 02/02606. {GenBank accession number: gi|4376736|gb|AAD18595.1 'CPn0453'; SEQ ID NO: 29 below}.

SEQ ID No 29

```
  1  MKTSIRKFLI STTLAPCFAS TAFTVEVIMP SENFDGSSGK IFPYTTLSDP
 51  RGTLCIFSGD LYIANLDNAI SRTSSSCFSN RAGALQILGK GGVFSFLNIR
101  SSADGAAISS VITQNPELCP LSFSGFSQMI FDNCESLTSD TSASNVIPHA
151  SAIYATTPML FTNNDSILFQ YNRSAGFGAA IRGTSITIEN TKKSLLFNGN
201  GSISNGGALT GSAAINLINN SAPVIFSTNA TGIYGGAIYL TGGSMLTSGN
251  LSGVLFVNNS SRSGGAIYAN GNVTFSNNSD LTFQNNTASP QNSLPAPTPP
301  PTPPAVTPLL GYGGAIFCTP PATPPPTGVS LTISGENSVT FLENIASEQG
351  GALYGKKISI DSNKSTIFLG NTAGKGGAIA IPESGELSLS ANQGDILFNK
401  NLSITSGTPT RNSIHFGKDA KFATLGATQG YTLYFYDPIT SDDLSAASAA
451  ATVVVNPKAS ADGAYSGTIV FSGETLTATE AATPANATST LNQKLELEGG
501  TLALRNGATL NVHNFTQDEK SVVIMDAGTT LATTNGANNT DGAITLNKLV
551  INLDSLDGTK AAVVNVQSTN GALTISGTLG LVKNSQDCCD NHGMFNKDLQ
601  QVPILELKAT SNTVTTTDFS LGTNGYQQSP YGYQGTWEFT IDTTTHTVTG
651  NWKKTGYLPH PERLAPLIPN SLWANVIDLR AVSQASAADG EDVPGKQLSI
701  TGITNFFHAN HTGDARSYRH MGGGYLINTY TRITPDAALS LGFGQLFTKS
751  KDYLVGHGHS NVYFATVYSN ITKSLFGSSR FFSGGTSRVT YSRSNEKVKT
801  SYTKLPKGRC SWSNNCWLGE LEGNLPITLS SRILNLKQII PFVKAEVAYA
851  THGGIQENTP EGRIFGHGHL LNVAVPVGVR FGKNSHNRPD FYTIIVAYAP
901  DVYRHNPDCD TTLPINGATW TSIGNNLTRS TLLVQASSHT SVNDVLEIFG
951  HCGCDIRRTS RQYTLDIGSK LRF*
```

Pmp 14 (CPn0454)

One example of a Pmp 14 protein is set forth as SEQ ID NO[s] 35 & 36 in WO 02/02606. {GenBank accession number: gi|4376737|gb|AAD18596.1 'CPn0454'; SEQ ID NO: 30 below}.

SEQ ID No 30

```
   1  MPLSFKSSSF CLLACLCSAS CAFAETRLGG NFVPPITNQG EEILLTSDFV
  51  CSNFLGASFS SSFINSSSNL SLLGKGLSLT FTSCQAPTNS NYALLSAAET
 101  LTFKNFSSIN FTGNQSTGLG GLIYGKDIVF QSIKDLIFTT NRVAYSPASV
 151  TTSATPAITT VTTGASALQP TDSLTVENIS QSIKFFGNLA NFGSAISSSP
 201  TAVVKFINNT ATMSFSHNFT SSGGGVIYGG SSLLFENNSG CIIFTANSCV
 251  NSLKGVTPSS GTYALGSGGA ICIPTGTFEL KNNQGKCTFS YNGTPNDAGA
 301  IYAETCNIVG NQGALLLDSN TAARNGGAIC AKVLNIQGRG PIEFSRNRAE
 351  KGGAIFIGPS VGDPAKQTST LTILASEGDI AFQGNMLNTK PGIRNAITVE
 401  AGGEIVSLSA QGGSRLVFYD PITHSLPTTS PSNKDITINA NGASGSVVFT
 451  SKGLSSTELL LPANTTTILL GTVKIASGEL KITDNAVVNV LGFATQGSGQ
 501  LTLGSGGTLG LATPTGAPAA VDFTIGKLAF DPFSFLKRDF VSASVNAGTK
 551  NVTLTGALVL DEHDVTDLYD MVSLQTPVAI PIAVFKGATV TKTGFPDGEI
 601  ATPSHYGYQG KWSYTWSRPL LIPAPDGGFP GGPSPSANTL YAVWNSDTLV
 651  RSTYILDPER YGEIVSNSLW ISFLGNQAFS DILQDVLLID HPGLSITAKA
 701  LGAYVEHTPR QGHEGFSGRY GGYQAALSMN YTDHTTLGLS FGQLYGKTNA
 751  NPYDSRCSEQ MYLLSFFGQF PIVTQKSEAL ISWKAAYGYS KNHLNTTYLR
 801  PDKAPKSQGQ WHNNSYYVLI SAEHPFLNWC LLTRPLAQAW DLSGFISAEF
 851  LGGWQSKFTE TGDLQRSFSR GKGYNVSLPI GCSSQWFTPF KKAPSTLTIK
 901  LAYKPDIYRV NPHNIVTVVS NQESTSISGA NLRRHGLFVQ IHDVVDLTED
 951  TQAFLNYTFD GKNGFTNHRV STGLKSTF*
```

Pmp 15 (CPn0466)

One example of a Pmp 15 protein is set forth as SEQ ID NO[s] 5 & 6 in WO 02/02606. {GenBank accession number: gi|4376751|gb|AAD18608.1 'CPn0466'; SEQ ID NO: 31 below}.

SEQ ID No 31

```
   1  MRFFCFGMLL PFTFVLANEG LQLPLETYIT LSPEYQAAPQ VGFTHNQNQD
  51  LAIVGNHNDF ILDYKYYRSN GGALTCKNLL ISENIGNVFF EKNVCPNSGG
 101  AIYAAQNCTI SKNQNYAFTT NLVSDNPTAT AGSLLGGALF AINCSITNNL
 151  GQGTFVDNLA LNKGGALYTE TNLSIKDNKG PIIIKQNRAL NSDSLGGGIY
 201  SGNSLNIEGN SGAIQITSNS SGSGGGIFST QTLTISSNKK LIEISENSAF
 251  ANNYGSNFNP GGGGLTTTFC TILNNREGVL FNNNQSQSNG GAIHAKSIII
 301  KENGPVYFLN NTATRGGALL NLSAGSGNGS FILSADNGDI IFNNNTASKH
 351  ALNPPYRNAI HSTPNMNLQI GARPGYRVLF YDPIEHELPS SFPILFNFET
 401  GHTGTVLFSG EHVHQNFTDE MNFFSYLRNT SELRQGVLAV EDGAGLACYK
 451  FFQRGGTLLL GQGAVITTAG TIPTPSSTPT TVGSTITLNH IAIDLPSILS
 501  FQAQAPKIWI YPTKTGSTYT EDSNPTITIS GTLTLRNSNN EDPYDSLDLS
 551  HSLEKVPLLY IVDVAAQKIN SSQLDLSTLN SGEHYGYQGI WSTYWVETTT
 601  ITNPTSLLGA NTKHKLLYAN WSPLGYRPHP ERRGEFITNA LWQSAYTALA
 651  GLHSLSSWDE EKGHAASLQG IGLLVHQKDK NGFKGFRSHM TGYSATTEAT
 701  SSQSPNFSLG FAQFFSKAKE HESQNSTSSH HYFSGMCIEN TLFKEWIRLS
 751  VSLAYMFTSE HTHTMYQGLL EGNSQGSFHN HTLAGALSCV FLPQPHGESL
 801  QIYPFITALA IRGNLAAFQE SGDHAREFSL HRPLTDVSLP VGIRASWKNH
 851  HRVPLVWLTE ISYRSTLYRQ DPELHSKLLI SQGTWTTQAT PVTYNALGIK
 901  VKNTMQVFPK VTLSLDYSAD ISSSTLSHYL NVASRMRF*
```

Pmp 16 (CPn0467)

One example of a Pmp 16 protein is set forth as SEQ ID NO[s] 7 & 8 in WO 02/02606. {GenBank accession number: gi|4376752|gb|AAD18609.1| 'CPn0467'; SEQ ID NO: 32 below}.

SEQ ID No 32

```
   1  MFGMTPAVYS LQTDSLEKFA LERDEEFRTS FPLLDSLSTL TGFSPITTFV
```

```
  51  GNRHNSSQDI VLSNYKSIDN ILLLWTSAGG AVSCNNFLLS NVEDHAFFSK
 101  NLAIGTGGAI ACQGACTITK NRGPLIFFSN RGLNNASTGG ETRGGAIACN
 151  GDFTISQNQG TFYFVNNSVN NWGGALSTNG HCRIQSNRAP LLFFNNTAPS
 201  GGGALRSENT TISDNTRPIY FKNNCGNNGG AIQTSVTVAI KNNSGSVIFN
 251  NNTALSGSIN SGNGSGGAIY TTNLSIDDNP GTILFNNNYC IRDGGAICTQ
 301  FLTIKNSGHV YFTNNQGNWG GALMLLQDST CLLFAEQGNI AFQNNEVFLT
 351  TFGRYNAIHC TPNSNLQLGA NKGYTTAFFD PIEHQHPTTN PLIFNPNANH
 401  QGTILFSSAY IPEASDYENN FISSSKNTSE LRNGVLSIED RAGWQFYKFT
 451  QKGGILKLGH AASIATTANS ETPSTSVGSQ VIINNLAINL PSILAKGKAP
 501  TLWIRPLQSS APFTEDNNPT ITLSGPLTLL NEENRDPYDS IDLSEPLQNI
 551  HLLSLSDVTA RHINTDNFHP ESLNATEHYG YQGIWSPYWV ETITTTNNAS
 601  IETANTLYRA LYANWTPLGY KVNPEYQGDL ATTPLWQSFH TMFSLLRSYN
 651  RTGDSDIERP FLEIQGIADG LFVHQNSIPG APGFRIQSTG YSLQASSETS
 701  LHQKISLGFA QFFTRTKEIG SSNNVSAHNT VSSLYVELPW FQEAFATSTV
 751  LAYGYGDHHL HSLHPSHQEQ AEGTCYSHTL AAAIGCSFPW QQKSYLHLSP
 801  FVQAIAIRSH QTAFEEIGDN PRKFVSQKPF YNLTLPLGIQ GKWQSKFHVP
 851  TEWTLELSYQ PVLYQQNPQI GVTLLASGGS WDILGHNYVR NALGYKVHNQ
 901  TALFRSLDLF LDYQGSVSSS TSTHHLQAGS TLKF*
```

Pmp 18 (CPn0471)

One example of a Pmp 18 protein is set forth as SEQ ID No 33 below{GenBank accession number: gi|4376753|gb|AAD18610.1| 'CPn0471'.

SEQ ID No 33

```
  1 MQNNRSLSKS SFFVGALILG KTTILLNATP LSDYFDNQAN QLTTLFPLID TLTNMTPYSH
 61 RATLFGVRDD TNQDIVLDHQ NSIESWFENF SQDGGALSCK SLAITNTKNQ ILFLNSFAIK
121 RAGAMYVNGN FDLSENHGSI IFSGNLSFPN ASNFADTCTG GAVLCSKNVT ISKNQGTAYF
181 INNKAKSSGG AIQAAIINIK DNTGPCLFFN NAAGGTAGGA LFANACRIEN NSQPIYFLNN
241 QSGLGGAIRV HQECILTKNT GSVIFNNNFA MEADISANHS SGGAIYCISC SIKDNPGIAA
301 FDNNTAARDG GAICTQSLTI QDSGPVYFTN NQGTWGGAIM LRQDGACTLF ADQGDIIFYN
361 NRHFKDTFSN HVSVNCTRNV SLTVGASQGH SATFYDPILQ RYTIQNSIQK FNPNPEHLGT
421 ILFSSTYIPD TSTSRDDFIS HFRNHIGLYN GTLALEDRAE WKVYKFDQFG GTLRLGSRAV
481 FSTTDEEQSS SSVGSVININ NLAINLPSIL GNRVAPKLWI RPTGSSAPYS EDNNPIINLS
541 GPLSLLDDEN LDPYDTADLA QPIAEVPLLY LLDVTAKHIN TDNFYPEGLN TTQHYGYQGV
601 WSPYWIETIT TSDTSSEDTV NTLHRQLYGD WTPTGYKVNP ENKGDIALSA FWQSFHNLFA
661 TLRYQTQQGQ IAPTASGEAT RLFVHQNSNN DAKGFHMEAT GYSLGTTSNT ASNHSFGVNF
721 SQLFSNLYES HSDNSVASHT TTVALQINNP WLQERFSTSA SLAYSYSNHH IKASGYSGKI
781 QTEGKCYSTT LGAALSCSLS LQWRSRPLHF TPFIQAIAVR SNQTAFQESG DKARKFSVHK
841 PLYNLTVPLG IQSAWESKFR LPTYWNIELA YQPVLYQQNP EINVSLESSG SSWLLSGTTL
901 ARNAIAFKGR NQIFIFPKLS VFLDYQGSVS SSTTTHYLHA GTTFKF
```

Pmp 19 (CPn0539)

One example of a Pmp 19 protein is set forth as SEQ ID No 34 below {GenBank accession number: gi|4376829|gb|AAD18679.1 'CPn0539'; SEQ ID NO: 34 below}.

SEQ ID No 34

```
  1 MKQMRLWGFL FLSSFCQVSY LRANDVLLPL SGIHSGEDLE LFTLRSSSPT KTTYSLRKDF
 61 IVCDFAGNSI HKPGAAFLNL KGDLFFINST PLAALTFKNI HLGARGAGLF SESNVTFKGL
121 HSLVLENNES WGGVLTTSGD LSFINNTSVL CQNNISYGPG GALLLQGRKS KALFFRDNRG
181 TILFLKNKAV NQDESHPGYG GAVSSISPGS PITFADNQEI LFQENEGELG GAIYNDQGAI
241 TFENNFQTTS FFSNKASFGG AVYSRYCNLY SQWGDTLFTK NAAAKVGGAI HADYVHIRDC
301 KGSIVFEENS ATAGGAIAVN AVCDINAQGP VRFINNSALG LNGGAIYMQA TGSILRLHAN
361 QGDIEFCGNK VRSQFHSHIN STSNFTNNAI TIQGAPREFS LSANEGHRIC FYDPIISATE
421 NYNSLYINHQ RLLEAGGAVI FSGARLSPEH KKENKNKTSI INQPVRLCSG VLSIEGGAIL
481 AVRSFYQEGG LLALGPGSKL TTQGKNSEKD KIVITNLGFN LENLDSSDPA EIRATEKASI
541 EISGVPRVYG HTESFYENHE YASKPYTTSI ILSAKKLVTA PSRPEKDIQN LIIAESEYMG
601 YGYQGSWEFS WSPNDTKEKK TIIASWTPTG EFSLDPKRRG SFIPTTLWST FSGLNIASNI
661 VNNNYLNNSE VIPLQHLCVF GGPVYQIMEQ NPKQSSNNLL VQHAGHNVGA RIPFSFNTIL
721 SAALTQLFSS SSQQNVADKS HAQILIGTVS LNKSWQALSL RSSFSYTEDS QVMKHVFPYK
781 GTSRGSWRNY GWSGSVGMSY AYPKGIRYLK MTPFVDLQYT KLVQNPFVET GYDPRYFSSS
```

```
841 EMTNLSLPIG IALEMRFIGS RSSLFLQVST SYIKDLRRVN PQSSASLVLN HYTWDIQGVP
901 LGKEALNITL NSTIKYKIVT AYMGISSTQR EGSNLSANAH AGLSLSF
```

As the Examples demonstrate, we and others have demonstrated (Grimwood et al (2001), Infection and Immunity 69(4), 2383-2389) using Flow cytometry (FACS) analyses and Western Blot analyses that PMP19 does not appear to be surface exposed. However, high levels of mRNA expression is nevertheless observed in gene microarray analysis of pmp19 (CPn0539).

Pmp 20 (CPn0540)

One example of a Pmp 20 protein is set forth as SEQ ID NO[s] 119 & 120 in WO 02/02606. {GenBank accession number: gi|4376830|gb|AAD18680.1 'CPn0540'; SEQ ID NO: 35 below}.

SEQ ID No 35

```
   1 MKWLPATAVF AAVLPALTAF GDPASVEIST SHTGSGDPTS DAALTGFTQS
  51 STETDGTTYT IVGDITFSTF TNIPVPVVTP DANDSSSNSS KGGSSSSGAT
 101 SLIRSSNLHS DFDFTKDSVL DLYHLFFPSA SNTLNPALLS SSSSGGSSSS
 151 SSSSSSGSAS AVVAADPKGG AAFYSNEANG TLTFTTDSGN PGSLTLQNLK
 201 MTGDGAAIYS KGPLVFTGLK NLTFTGNESQ KSGGAAYTEG ALTTQAIVEA
 251 VTFTGNTSAG QGGAIYVKEA TLFNALDSLK FEKNTSGQAG GGIYTESTLT
 301 ISNITKSIEF ISNKASVPAP APEPTSPAPS SLINSTTIDT STLQTRAASA
 351 TPAVAPVAAV TPTPISTQET AGNGGAIYAK QGISISTFKD LTFKSNSASV
 401 DATLTVDSST IGESGGAIFA ADSIQIQQCT GTTLFSGNTA NKSGGGIYAV
 451 GQVTLEDIAN LKMTNNTCKG EGGAIYTKKA LTINNGAILT TFSGNTSTDN
 501 GGAIFAVGGI TLSDLVEVRF SKNKTGNYSA PITKAASNTA PVVSSSTTAA
 551 SPAVPAAAAA PVTNAAKGGA LYSTEGLTVS GITSILSFEN NECQNQGGGA
 601 YVTKTFQCSD SHRLQFTSNK AADEGGGLYC GDDVTLTNLT GKTLFQENSS
 651 EKHGGGLSLA SGKSLTMTSL ESFCLNANTA KENGGGANVP ENIVLTFTYT
 701 PTPNEPAPVQ QPVYGEALVT GNTATKSGGG IYTKNAAFSN LSSVTFDQNT
 751 SSENGGALLT QKAADKTDCS FTYITNVNIT NNTATGNGGG IAGGKAHFDR
 801 IDNLTVQSNQ AKKGGGVYLE DALILEKVIT GSVSQNTATE SGGGIYAKDI
 851 QLQALPGSFT ITDNKVETSL TTSTNLYGGG IYSSGAVTLT NISGTFGITG
 901 NSVINTATSQ DADIQGGGIY ATTSLSINQC NTPILFSNNS AATKKTSTTK
 951 QIAGGAIFSA AVTIENNSQP IIFLNNSAKS EATTAATAGN KDSCGGAIAA
1001 NSVTLTNNPE ITFKGNYAET GGAIGCIDLT NGSPPRKVSI ADNGSVLFQD
1051 NSALNRGGAI YGETIDISRT GATFIGNSSK HDGSAICCST ALTLAPNSQL
1101 IFENNKVTET TATTKASINN LGAAIYGNNE TSDVTISLSA ENGSIFFKNN
1151 LCTATNKYCS IAGNVKFTAI EASAGKAISF YDAVNVSTKE TNAQELKLNE
1201 KATSTGTILF SGELHENKSY IPQKVTFAHG NLILGKNAEL SVVSFTQSPG
1251 TTITMGPGSV LSNHSKEAGG IAINNVIIDF SEIVPTKDNA TVAPPTLKLV
1301 SRTNADSKDK IDITGTVTLL DPNGNLYQNS YLGEDRDITL FNIDNSASGA
1351 VTATNVTLQG NLGAKKGYLG TWNLDPNSSG SKIILKWTFD KYLRWPYIPR
1401 DNHFYINSIW GAQNSLVTVK QGILGNMLNN ARFEDPAFNN FWASAIGSFL
1451 RKEVSRNSDS FTYHGRGYTA AVDAKPRQEF ILGAAFSQVF GHAESEYHLD
1501 NYKHKGSGHS TQASLYAGNI FYFPAIRSRP ILFQGVATYG YMQHDTTTYY
1551 PSIEEKNMAN WDSIAWLFDL RFSVDLKEPQ PHSTARLTFY TEAEYTRIRQ
1601 EKFTELDYDP RSFSACSYGN LAIPTGFSVD GALAWREIIL YNKVSAAYLP
1651 VILRNNPKAT YEVLSTKEKG NVVNVLPTRN AARAEVSSQI YLGSYWTLYG
1701 TYTIDASMNT LVQMANGGIR FVF*
```

Pmp21 (CPn0963)

One example of a Pmp 21 protein is set forth as SEQ ID NO[s] 83 & 84 in WO 02/02606. {GenBank accession number: gi|4377287|gb|AAD19099.1| 'CPn0963'; SEQ ID NO: 36 below}.

SEQ ID No 36

```
   1 MVAKKTVRSY RSSFSHSVIV AILSAGIAFE AHSLHSSELD LGVFNKQFEE
```

```
  51  HSAHVEEAQT SVLKGSDPVN PSQKESEKVL YTQVPLTQGS SGESLDLADA
 101  NFLEHFQHLF EETTVFGIDQ KLVWSDLDTR NFSQPTQEPD TSNAVSEKIS
 151  SDTKENRKDL ETEDPSKKSG LKEVSSDLPK SPETAVAAIS EDLEISENIS
 201  ARDPLQGLAF FYKNTSSQSI SEKDSSFQGI IFSGSGANSG LGFENLKAPK
 251  SGAAVYSDRD IVFENLVKGL SFISCESLED GSAAGVNIVV THCGDVTLTD
 301  CATGLDLEAL RLVKDFSRGG AVFTARNHEV QNNLAGGILS VVGNKGAIVV
 351  EKNSAEKSNG GAFACGSFVY SNNENTALWK ENQALSGGAI SSASDIDIQG
 401  NCSAIEFSGN QSLIALGEHI GLTDFVGGGA LAAQGTLTLR NNAVVQCVKN
 451  TSKTHGGAIL AGTVDLNETI SEVAFKQNTA ALTGGALSAN DKVIIANNFG
 501  EILFEQNEVR NHGGAIYCGC RSNPKLEQKD SGENINIIGN SGAITFLKNK
 551  ASVLEVMTQA EDYAGGGALW GHNVLLDSNS GNIQFIGNIG GSTFWIGEYV
 601  GGGAILSTDR VTISNNSGDV VFKGNKGQCL AQKYVAPQET APVESDASST
 651  NKDEKSLNAC SHGDHYPPKT VEEEVPPSLL EEHPVVSSTD IRGGGAILAQ
 701  HIFITDNTGN LRFSGNLGGG EESSTVGDLA IVGGGALLST NEVNVCSNQN
 751  VVFSDNVTSN GCDSGGAILA KKVDISANHS VEFVSNGSGK FGGAVCALNE
 801  SVNITDNGSA VSFSKNRTRL GGAGVAAPQG SVTICGNQGN IAFKENFVFG
 851  SENQRSGGGA IIANSSVNIQ DNAGDILFVS NSTGSYGGAI FVGSLVASEG
 901  SNPRTLTITG NSGDILFAKN STQTAASLSE KDSFGGGAIY TQNLKIVKNA
 951  GNVSFYGNRA PSGAGVQIAD GGTVCLEAFG GDILFEGNIN FDGSFNAIHL
1001  CGNDSKIVEL SAVQDKNIIF QDAITYEENT IRGLPDKDVS PLSAPSLIFN
1051  SKPQDDSAQH HEGTIRFSRG VSKIPQIAAI QEGTLALSQN AELWLAGLKQ
1101  ETGSSIVLSA GSILRIFDSQ VDSSAPLPTE NKEETLVSAG VQINMSSPTP
1151  NKDKAVDTPV LADIISITVD LSSFVPEQDG TLPLPPEIII PKGTKLHSNA
1201  IDLKIIDPTN VGYENHALLS SHKDIPLISL KTAEGMTGTP TADASLSNIK
1251  IDVSLPSITP ATYGHTGVWS ESKMEDGRLV VGWQPTGYKL NPEKQGALVL
1301  NNLWSHYTDL RALKQEIFAH HTIAQRMELD FSTNVWGSGL GVVEDCQNIG
1351  EFDGFKHHLT GYALGLDTQL VEDFLIGGCF SQFFGKTESQ SYKAKNDVKS
1401  YMGAAYAGIL AGPWLIKGAF VYGNINNDLT TDYGTLGIST GSWIGKGFIA
1451  GTSIDYRYIV NPRRFISAIV STVVPFVEAE YVRIDLPEIS EQGKEVRTFQ
1501  KTRFENVAIP FGFALEHAYS RGSRAEVNSV QLAYVFDVYR KGPVSLITLK
1551  DAAYSWKSYG VDIPCKAWKA RLSNNTEWNS YLSTYLAFNY EWREDLIAYD
1601  FNGGIRIIF*
```

Preferred PMP proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to one of the polypeptide sequences set forth for the pmp proteins above and/or (b) which is a fragment of at least *n* consecutive amino acids of one of the polypeptide sequences set forth above wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PMP proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of the polypeptide sequences set forth above. Preferred fragments of (b) comprise an epitope from one of the polypeptide sequences set forth above. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of one of the polypeptide sequences set forth above. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

Fifth Antigen Group

The immunogenicity of other *Chlamydia pneumoniae* antigens may be improved by combination with two or more *Chlamydia pneumoniae* antigens from either the first antigen group or the second antigen group or the third antigen group or the fourth antigen group. Such other *Chlamydia pneumoniae* antigens include a fifth antigen group consisting one or more cell surface exposed proteins. These antigens are referred to herein as the "fifth antigen group". Each of the *Chlamydia pneumoniae* antigens of the fifth antigen group is described in more detail below.

(37) PorB Outer Membrane Protein B (CPn0854)

One example of a PorB protein is set forth as SEQ ID NO[s]: 67 & 68 in WO 02/02606. {GenBank accession number: gi|4377170|gb|AAD18992.1| 'CPn0854'; SEQ ID NO: 37 below}. Preferred PorB proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 37; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 37, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PorB proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 37. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 37. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 37. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 37

```
  1  MNSKMLKHLR LATLSFSMFF GIVSSPAVYA LGAGNPAAPV LPGVNPEQTG
 51  WCAFQLCNSY DLFAALAGSL KFGFYGDYVF SESAHITNVP VITSVTTSGT
101  GTTPTITSTT KNVDFDLNNS SISSSCVFAT IALQETSPAA IPLLDIAFTA
151  RVGGLKQYYR LPLNAYRDFT SNPLNAESEV TDGLIEVQSD YGIVWGLSLQ
201  KVLWKDGVSF VGVSADYRHG SSPINYIIVY NKANPEIYFD ATDGNLSYKE
251  WSASIGISTY LNDYVLPYAS VSIGNTSRKA PSDSFTELEK QFTNFKFKIR
301  KITNFDRVNF CFGTTCCISN NFYYSVEGRW GYQRAINITS GLQF*
```

(38) 76kDa Protein Homolog (CPn0728)

One example of a 76kDa Protein Homolog protein is set forth as SEQ ID NO[s]: 13 & 14 in WO 02/02606. {GenBank accession number: gi|4377033|gb|AAD18867.1| 'CPn0728'; SEQ ID NO: 38 below}. Preferred 76kDa proteins homologs for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 38; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 21, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These 76kDa protein homologs include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 38. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 38. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 38. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 38

```
  1  MVNPIGPGPI DETERTPPAD LSAQGLEASA ANKSAEAQRI AGAEAKPKES
 51  KTDSVERWSI LRSAVNALMS LADKLGIASS NSSSSTSRSA DVDSTTATAP
101  TPPPPTFDDY KTQAQTAYDT IFTSTSLADI QAALVSLQDA VTNIKDTAAT
151  DEETAIAAEW ETKNADAVKV GAQITELAKY ASDNQAILDS LGKLTSFDLL
201  QAALLQSVAN NNKAAELLKE MQDNPVVPGK TPAIAQSLVD QTDATATQIE
```

```
251  KDGNAIRDAY FAGQNASGAV ENAKSNNSIS NIDSAKAAIA TAKTQIAEAQ
301  KKFPDSPILQ EAEQMVIQAE KDLKNIKPAD GSDVPNPGTT VGGSKQQGSS
351  IGSIRVSMLL DDAENETASI LMSGFRQMIH MFNTENPDSQ AAQQELAAQA
401  RAAKAAGDDS AAAALADAQK ALEAALGKAG QQQGILNALG QIASAAVVSA
451  GVPPAAASSI GSSVKQLYKT SKSTGSDYKT QISAGYDAYK SINDAYGRAR
501  NDATRDVINN VSTPALTRSV PRARTEARGP EKTDQALARV ISGNSRTLGD
551  VYSQVSALQS VMQIIQSNPQ ANNEEIRQKL TSAVTKPPQF GYPYVQLSND
601  STQKFIAKLE SLFAEGSRTA AEIKALSFET NSLFIQQVLV NIGSLYSGYL
651  Q*
```

(39) OmpA conserved outer membrane protein (CPn0695)

One example of an OmpA conserved outer membrane protein protein is set forth as SEQ ID NO[s]: 59 & 60 in WO 02/02606. {GenBank accession number: gi|4376998|gb|AAD18834.1| 'CPn0695'; SEQ ID NO: 39 below}. Preferred ompA proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 39; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 39, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 39. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 39. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 39. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 39

```
  1  MKKLLKSALL SAAFAGSVGS LQALPVGNPS DPSLLIDGTI WEGAAGDPCD
 51  PCATWCDAIS LRAGFYGDYV FDRILKVDAP KTFSMGAKPT GSAAANYTTA
101  VDRPNPAYNK HLHDAEWFTN AGFIALNIWD RFDVFCTLGA SNGYIRGNST
151  AFNLVGLFGV KGTTVNANEL PNVSLSNGVV ELYTDTSFSW SVGARGALWE
201  CGCATLGAEF QYAQSKPKVE ELNVICNVSQ FSVNKPKGYK GVAFPLPTDA
251  GVATATGTKS ATINYHEWQV GASLSYRLNS LVPYIGVQWS RATFDADNIR
301  IAQPKLPTAV LNLTAWNPSL LGNATALSTT DSFSDFMQIV SCQINKFKSR
351  KACGVTVGAT LVDADKWSLT AEARLINERA AHVSGQFRF*
```

(40) PepA (CPn0385)

One example of a PepA protein protein is set forth as SEQ ID NO[s]: 99 & 100 in WO 02/02606. {GenBank accession number: gi|4376664|gb|AAD18529.1 'CPn0385'; SEQ ID NO: 40 below}. Preferred PepA proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 40; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 40, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PepA proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 40 Preferred fragments of (b) comprise an epitope from SEQ ID NO: 40. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 40. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 40

```
   1  MVLFHAQASG RNRVKADAIV LPFWHFKDAK NAASFEAEFE PSYLPALENF
  51  QGKTGEIELL YSSPKAKEKR IVLLGLGKNE ELTSDVVFQT YATLTRVLRK
 101  AKCSTVNIIL PTISELRLSA EEFLVGLSSG ILSLNYDYPR YNKVDRNLET
 151  PLSKVTVIGI VPKMADAIFR KEAAIFEGVY LTRDLVNRNA DEITPKKLAE
 201  VALNLGKEFP SIDTKVLGKD AIAKEKMGLL LAVSKGSCVD PHFIVVRYQG
 251  RPKSKDHTVL IGKGVTFDSG GLDLKPGKSM LTMKEDMAGG ATVLGILSAL
 301  AVLELPINVT GIIPATENAI DGASYKMGDV YVGMSGLSVE ICSTDAEGRL
 351  ILADAITYAL KYCKPTRIID FATLTGAMVV SLGEEVAGFF SNNDVLAEDL
 401  LEASAETSEP LWRLPLVKKY DKTLHSDIAD MKNLGSNRAG AITAALFLQR
 451  FLEESSVAWA HLDIAGTAYH EKEEDRYPKY ASGFGVRSIL YYLENSLSK*
```

(41) Conserved Outer Membrane Protein (Cpn0278)

One example of a conserved outer membrane protein protein is set forth as SEQ ID NO: 41 below. GenBank Accession No. GI:4376552; AAD18427.1. Preferred conserved outer membrane proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 41; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 41, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These conserved outer membrane proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 41. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 41. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 41. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 41

```
   1 MKKKLSLLVG LIFVLSSCHK EDAQNKIRIV ASPTPHAELL ESLQEEAKDL GIKLKILPVD
  61 DYRIPNRLLL DKQVDANYFQ HQAFLDDECE RYDCKGELVV IAKVHLEPQA IYSKKHSSLE
 121 RLKSQKKLTI AIPVDRTNAQ RALHLLEECG LIVCKGPANL NMTAKDVCGK ENRSINILEV
 181 SAPLLVGSLP DVDAAVIPGN FAIAANLSPK KDSLCLEDLS VSKYTNLVVI RSEDVGSPKM
 241 IKLQKLFQSP SVQHFFDTKY HGNILTMTQD NG
```

Sixth Antigen Group

The immunogenicity of other *Chlamydia pneumoniae* antigens may be improved by combination with two or more *Chlamydia pneumoniae* antigens from either the first antigen group or the second antigen group or the third antigen group or the fourth antigen group or the fifth antigen group. Such other *Chlamydia pneumoniae* antigens include a sixth antigen group consisting one or more FACS positive CPn antigens. These antigens are referred to herein as the " sixth antigen group". Each of the *Chlamydia pneumoniae* antigens of the sixth antigen group is described in more detail below.

(42) Predicted Omp (CPn0020)

One example of a predicted Omp protein is set forth as SEQ ID NO[s]: 91 & 92 in WO 02/02606. {GenBank accession number gi|4376272|gb|AAD18173.1: 'CPn0020';

SEQ ID NO: 42 below). Preferred Omp proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 42; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 42, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Omp proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 42. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 42. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 42. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 42

```
   1  MKRCFLFLAS FVLMGSSADA LTHQEAVKKK NSYLSHFKSV SGIVTIEDGV
  51  LNIHNNLRIQ ANKVYVENTV GQSLKLVAHG NVMVNYRAKT LVCDYLEYYE
 101  DTDSCLLTNG RFAMYPWFLG GSMITLTPET IVIRKGYIST SEGPKKDLCL
 151  SGDYLEYSSD SLLSIGKTTL RVCRIPILFL PPFSIMPMEI PKPPINFRGG
 201  TGGFLGSYLG MSYSPISRKH FSSTFFLDSF FKHGVGMGFN LHCSQKQVPE
 251  NVFNMKSYYA HRLAIDMAEA HDRYRLHGDF CFTHKHVNFS GEYHLSDSWE
 301  TVADIFPNNF MLKNTGPTRV DCTWNDNYFE GYLTSSVKVN SFQNANQELP
 351  YLTLRQYPIS IYNTGVYLEN IVECGYLNFA FSDHIVGENF SSLRLAARPK
 401  LHKTVPLPIG TLSSTLGSSL IYYSDVPEIS SRHSQLSAKL QLDYRFLLHK
 451  SYIQRRHIIE PFVTFITETR PLAKNEDHYI FSIQDAFHSL NLLKAGIDTS
 501  VLSKTNPRFP RIHAKLWTTH ILSNTESKPT FPKTACELSL PFGKKNTVSL
 551  DAEWIWKKHC WDHMNIRWEW IGNDNVAMTL ESLHRSKYSL IKCDRENFIL
 601  DVSRPIDQLL DSPLSDHRNL ILGKLFVRPH PCWNYRLSLR YGWHRQDTPN
 651  YLEYQMILGT KIFEHWQLYG VYERREADSR FFFFLKLDKP KKPPF*
```

(43) Predicted Omp (CPn0021)

One example of a predicted Omp protein is set forth as SEQ ID NO[s]: 49 & 50 in WO 02/02606. {GenBank accession numbe gi|4376273|gb|AAD18174.1: 'CPn0021'; SEQ ID NO: 43 below}. Preferred Omp proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 43; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 43, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 43. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 43. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 43. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 43

```
   1  MGLFHLTLFG LLLCSLPISL VAKFPESVGH KILYISTQST QQALATYLEA
  51  LDAYGDHDFF VLRKIGEDYL KQSIHSSDPQ TRKSTIIGAG LAGSSEALDV
 101  LSQAMETADP LQQLLVLSAV SGHLGKTSDD LLFKALASPY PVIRLEAAYR
 151  LANLKNTKVI DHLHSFIHKL PEEIQCLSAA IFLRLETEES DAYIRDLLAA
```

```
201  KKSAIRSATA LQIGEYQQKR FLPTLRNLLT SASPQDQEAI LYALGKLKDG
251  QSYYNIKKQL QKPDVDVTLA AAQALIALGK EEDALPVIKK QALEERPRAL
301  YALRHLPSEI GIPIALPIFL KTKNSEAKLN VALALLELGC DTPKLLEYIT
351  ERLVQPHYNE TLALSFSKGR TLQNWKRVNI IVPQDPQERE RLLSTTRGLE
401  EQILTFLFRL PKEAYLPCIY KLLASQKTQL ATTAISFLSH TSHQEALDLL
451  FQAAKLPGEP IIRAYADLAI YNLTKDPEKK RSLHDYAKKL IQETLLFVDT
501  ENQRPHPSMP YLRYQVTPES RTKLMLDILE TLATSKSSED IRLLIQLMTE
551  GDAKNFPVLA GLLIKIVE*
```

(44) Oligopeptide Binding Protein Oppa-1 Lipoprotein (CPn0195)

One example of an oligopeptide binding protein is set forth as SEQ ID NO[s]: 23 and 24 in WO 02/02606. {GenBank accession number gi|4376466|gb|AAD18348.1: 'CPn0195'; SEQ ID NO: 44 below}. Preferred oligopeptide binding proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 44; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 44, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 44. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 44. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 44. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 44

```
1    MRKISVGICI TILLSLSVVL QGCKESSHSS TSRGELAINI RDEPRSLDPR
51   QVRLLSEISL VKHIYEGLVQ ENNLSGNIEP ALAEDYSLSS DGLTYTFKLK
101  SAFWSNGDPL TAEDFIESWK QVATQEVSGI YAFALNPIKN VRKIQEGHLS
151  IDHFGVHSPN ESTLVVTLES PTSHFLKLLA LPVFFPVHKS QRTLQSKSLP
201  IASGAFYPKN IKQKQWIKLS KNPHYYNQSQ VETKTITIHF IPDANTAAKL
251  FNQGKLNWQG PPWGERIPQE TLSNLQSKGH LHSFDVAGTS WLTFNINKFP
301  LNNMKLREAL ASALDKEALV STIFLGRAKT ADHLLPTNIH SYPEHQKQEM
351  AQRQAYAKKL FKEALEELQI TAKDLEHLNL IFPVSSSASS LLVQLIREQW
401  KESLGFAIPI VGKEFALLQA DLSSGNFSLA TGGWFADFAD PMAFLTIFAY
451  PSGVPPYAIN HKDFLEILQN IEQEQDHQKR SELVSQASLY LETFHIIEPI
501  YHDAFQFAMN KKLSNLGVSP TGVVDFRYAK EN*
```

(45) CHLPS 43 kDa Protein Homologue-1 (CPn0562)

One example of a CHLPS protein is set forth as SEQ ID NO: 45 below. GenBank Accession No. GI:4376854; AAD18702.1. Preferred CHLPS proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 45; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 45, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These CHLPS proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 45. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 45. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the

N-terminus of SEQ ID NO: 45. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 45

```
  1 MSIAIAREQY AAILDMHPKP SIAMFSSEQA RTSWEKRQAH PYLYRLLEII WGVVKFLLGL
 61 IFFIPLGLFW VLQKICQNFI LLGAGGWIFR PICRDSNLLR QAYAARLFSA SFQDHVSSVR
121 RVCLQYDEVF IDGLELRLPN AKPDRWMLIS NGNSDCLEYR TVLQGEKDWI FRIAEESQSN
181 ILIFNYPGVM KSQGNITRNN VVKSYQACVR YLRDEPAGPQ ARQIVAYGYS LGASVQAEAL
241 SKEIADGSDS VRWFVVKDRG ARSTGAVAKQ FIGSLGVWLA NLTHWNINSE KRSKDLHCPE
301 LFIYGKDSQG NLIGDGLFKK ETCFAAPFLD PKNLEECSGK KIPVAQTGLR HDHILSDDVI
361 KEVAGHIQRH FDN
```

(46) YscJ (Yop translocation J protein) (CPn0828)

One example of a YscJ protein is set forth as SEQ ID NO[s]: 109 and 110 in WO 02/02606. {GenBank accession number gi|4377140|gb|AAD18965.1| 'CPn0828'; SEQ ID NO: 46 below. Preferred YscJ proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 46; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 46, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These YscJ proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 46. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 46. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 46. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 46

```
  1 MVRRSISFCL FFLMTLLCCT SCNSRSLIVH GLPGREANEI VVLLVSKGVA
 51 AQKLPQAAAA TAGAATEQMW DIAVPSAQIT EALAILNQAG LPRMKGTSLL
101 DLFAKQGLVP SELQEKIRYQ EGLSEQMAST IRKMDGVVDA SVQISFTTEN
151 EDNLPLTASV YIKHRGVLDN PNSIMVSKIK RLIASAVPGL VPENVSVVSD
201 RAAYSDITIN GPWGLTEEID YVSVWGIILA KSSLTKFRLI FYVLILILFV
251 ISCGLLWVIW KTHTLIMTMG GTKGFFNPTP YTKNALEAKK AEGAAADKEK
301 KEDADSQGES KNAETSDKDS SDKDAPEGSN EIEGA*
```

(47) Hypothetical (CPn 0415)

One example of a hypothetical protein is set forth as SEQ ID NO[s]: 101 and 102 in WO 02/02606. {GenBank accession number gi|4376696|gb|AAD18559.1| 'CPn0415'; SEQ ID NO: 47 below}. Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 47; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 47, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 47. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 47. Other preferred fragments lack one or

more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 47. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 47

```
   1  MTLIFVIIIV WCNAFLIKLC VIMGLQSRLQ HCIEVSQNSN FDSQVKQFIY
  51  ACQDKTLRQS VLKIFRYHPL LKIHDIARAV YLLMALEEGE DLGLSFLNVQ
 101  QYPSGAVELF SCGGFPWKGL PYPAEHAEFG LLLLQIAEFY EESQAYVSKM
 151  SHFQQALFDH QGSVFPSLWS QENSRLLKEK TTLSQSFLFQ LGMQIHPEYS
 201  LEDPALGFWM QRTRSSSAFV AASGCQSSLG AYSSGDVGVI AYGPCSGDIS
 251  DCYYFGCCGI AKEFVCQKSH QTTEISFLTS TGKPHPRNTG FSYLRDSYVH
 301  LPIRCKITIS DKQYRVHAAL AEATSAMTFS IFCKGKNCQV VDGPRLRSCS
 351  LDSYKGPGND IMILGENDAI NIVSASPYME IFALQGKEKF WNADFLINIP
 401  YKEEGVMLIF EKKVTSEKGR FFTKMN*
```

(48) Hypothetical (CPn0514)

One example of a hypothetical protein is set forth as SEQ ID NO[s]: 87 and 88 in WO 02/02606. {GenBank accession number gi|4376802|gb|AAD18654.1| 'CPn0514'; SEQ ID NO: 48 below}. Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 48; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 48, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 48. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 48. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 48. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 48

```
   1  MSNQLQPCIS LGCVSYINSF PLSLQLIKRN DIRCVLAPPA DLLNLLIEGK
  51  LDVALTSSLG AISHNLGYVP GFGIAANQRI LSVNLYAAPT FFNSPQPRIA
 101  ATLESRSSIG LLKVLCRHLW RIPTPHILRF ITTKVLRQTP ENYDGLLLIG
 151  DAALQHPVLP GFVTYDLASG WYDLTKLPFV FALLLHSTSW KEHPLPNLAM
 201  EEALQQFESS PEEVLKEAHQ HTGLPPSLLQ EYYALCQYRL GEEHYESFEK
 251  FREYYGTLYQ QARL*
```

(49) Hypothetical (CPn0668)

One example of a hypothetical protein is set forth as SEQ ID NO[s]: 57 and 58 in WO 02/02606. {GenBank accession number gi|4376968|gb|AAD18807.1 'CPn0668'; SEQ ID NO: 49 below}. Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 49; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 49, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These

hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 49. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 49. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 49. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 49

```
   1  MKFLLYVPLL LVLVSTGCDA KPVSFEPFSG KLSTQRFEPQ HSAEEYFSQG
  51  QEFLKKGNFR KALLCFGIIT HHFPRDILRN QAQYLIGVCY FTQDHPDLAD
 101  KAFASYLQLP DAEYSEELFQ MKYAIAQRFA QGKRKRICRL EGFPKLMNAD
 151  EDALRIYDEI LTAFPSKDLG AQALYSKAAL LIVKNDLTEA TKTLKKLTLQ
 201  FPLHILSSEA FVRLSEIYLQ QAKKEPHNLQ YLHFAKLNEE AMKKQHPNHP
 251  LNEVVSANVG AMREHYARGL YATGRFYEKK KKAEAANIYY RTAITNYPDT
 301  LLVAKCQKRL DRISKHTS*
```

(50) Hypothetical (CPn0791)

One example of a hypothetical protein is set forth as SEQ ID NO[s]: 123 and 124 in WO 02/02606. {GenBank accession number gi|4377101|gb|AAD18929.1| 'CPn0791'; SEQ ID NO: 50 below}. Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 50; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 50, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 50. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 50. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 50. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 50

```
   1  MYSCYSKGIS HNYLLHPMSR LDIFVFDSLI ANQDQNLLEE IFCSEDTVLF
  51  KAYRTTALQS PLAAKNLNIA RKVANYILAD NGEIDTVKLV EAIHHLSQCT
 101  YPLGPHRHNE AQDREHLLKM LKALKENPKL KESIKTLFVP SYSTIQNLIR
 151  HTLALNPQTI LSTIHVRQAA LTALFTYLRQ DVGSCFATAP AILIHQEYPE
 201  RFLKDLNDLI SSGKLSRIVN QREIAVPINL SGCIGELFKP LRILDLYPDP
 251  LVKLSSSPGL KKAFSAANLI ETLGDSEAQI QQLLSHQYLM QKLQNVHETL
 301  TANDIIKSTL LHYYQLQEST VRAIFFKEGL FSKEQVAFST QHPRELSEIQ
 351  RVYHYLHAYE EAKSAFIHDT QNPLLKAWEY TLATLADASQ PTISNHIRLA
 401  LGWKSEDPHS LVSLVTHFVE EEVENIRILV QQCEQTYHEA RSQLEYIEGR
 451  MRNPLNNQDS QILTMDHMRF RQELNKALYE WDSAQEKAKK FLHLPEFLLS
 501  FYTKQIPLYF RSSYDAFIQE FAHLYANAPA GFRILFTHGR THPNTWSPIY
 551  SINEFIRFLS EFFTSTESEL LGKHAVINLE KETSRLVHNI TAMLHTDVFQ
 601  EALLTRILEA YQLPVPPSIL NHLDQLSQTP WVYVSGGTVD TLLLDYFESS
 651  EPLTLTEKHP ENPHELAAFY ADALKDLPTG IKSYLEEGSH SLLSSSPTHV
 701  FSIIAGSPLF REAWDNDWYS YTWLRDVWVK QHQDFLQDTI LPQLSIYAFI
 751  ENFCNKYALQ HVVHDFHDFC SDHSLTLPEL YDKGSRFLSS LFTKDKTVAL
 801  IYIRRLLYLM VREVPYVSEQ QLPEVLDNVS SYLGISSRIT YEKFRSLIEE
 851  TIPKMTLLSS ADLRHIYKGL LMQSYQKIYT EEDTYLRLTT AMRHHNLAYP
```

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901  APLLFADSNW PSIYFGFILN PGTTEIDLWK FNYAGLQGQP LDNIQELFAT
951  SRPWTLYANP IDYGMPPPPG YRSRLPKEFF *
```

(51) Hypothetical (CPn0792)

One example of a hypothetical protein is set forth as SEQ ID NO[s]: 61 and 62 in WO 02/02606. {GenBank accession number gi|4377102|gb|AAD18930.1| 'CPn0792'; SEQ ID NO: 51 below}. Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 51; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 51, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 51. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 51. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 51. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 51

```
   1  MKHTFTKRVL FFFFLVIPIP LLLNLMVVGF FSFSAAKANL VQVLHTRATN
  51  LSIEFEKKLT IHKLFLDRLA NTLALKSYAS PSAEPYAQAY NEMMALSNTD
 101  FSLCLIDPFD GSVRTKNPGD PFIRYLKQHP EMKKKLSAAV GKAFLLTIPG
 151  KPLLHYLILV EDVASWDSTT TSGLLVSFYP MSFLQKDLFQ SLHITKGNIC
 201  LVNKYGEVLF CAQDSESSFV FSLDLPNLPQ FQARSPSAIE IEKASGILGG
 251  ENLITVSINK KRYLGLVLNK IPIQGTYTLS LVPVSDLIQS ALKVPLNICF
 301  FYVLAFLLMW WIFSKINTKL NKPLQELTFC MEAAWRGNHN VRFEPQPYGY
 351  EFNELGNIFN CTLLLLLNSI EKADIDYHSG EKLQKELGIL SSLQSALLSP
 401  DFPTFPKVTF SSQHLRRRQL SGHFNGWTVQ DGGDTLLGII GLAGDIGLPS
 451  YLYALSARSL FLAYASSDVS LQKISKDTAD SFSKTTEGNE AVVAMTFIKY
 501  VEKDRSLELL SLSEGAPTMF LQRGESFVRL PLETHQALQP GDRLICLTGG
 551  EDILKYFSQL PIEELLKDPL NPLNTENLID SLTMMLNNET EHSADGTLTI
 601  LSFS*
```

(52) Hypothetical (CPn0820)

One example of a hypothetical protein is set forth as SEQ ID NO[s]: 113 and 114 in WO 02/02606. {GenBank accession number gi|4377132|gb|AAD18958.1| 'CPn0820'; SEQ ID NO: 52 below}. Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 52; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 52, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 52. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 52. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 52. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 52

```
   1  MCNSIAMKKQ KRGFVLMELL MSFTLIALLL GTLGFWYRKI YTVQKQKERI
  51  YNFYIEESRA YKQLRTLFSM SLSSSYEEPG SLFSLIFDRG VYRDPKLAGA
 101  VRASLHHDTK DQRLELRICN IKDQSYFETQ RLLSHVTHVV LSFQRNPDPE
 151  KLPETIALTI TREPKAYPPR TLTYQFAVGK*
```

(53) Hypothetical (CPn0126)

One example of a hypothetical protein is set forth as SEQ ID NO: 53 below. GenBank Accession No. GI:4376390; AAD18279.1 Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 53; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 53, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 53. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 53. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 53. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 53

```
   1 MVFSYYCMGL FFFSGAISSC GLLVSLGVGL GLSVLGVLLL LLAGLLLFKI QSMLREVPKA
  61 PDLLDLEDAS ERLRVKASRS LASLPKEISQ LESYIRSAAN DLNTIKTWPH KDQRLVETVS
 121 RKLERLAAAQ NYMISELCEI SEILEEEEHH LILAQESLEW IGKSLFSTFL DMESFLNLSH
 181 LSEVRPYLAV NDPRLLEITE ESWEVVSHFI NVTSAFKKAQ ILFKNNEHSR MKKKLESVQE
 241 LLETFIYKSL KRSYRELGCL SEKMRIIHDN PLFPWVQDQQ KYAHAKNEFG EIARCLEEFE
 301 KTFFWLDEEC AISYMDCWDF LNESIQNKKS RVDRDYISTK KIALKDRART YAKVLLEENP
 361 TTEGKIDLQD AQRAFERQSQ EFYTLEHTET KVRLEALQQC FSDLREATNV RQVRFTNSEN
 421 ANDLKESFEK IDKERVRYQK EQRLYWETID RNEQELREEI GESLRLQNRR KGYRAGYDAG
 481 RLKGLLRQWK KNLRDVEAHL EDATMDFEHE VSKSELCSVR ARLEVLEEEL MDMSPKVADI
 541 EELLSYEERC ILPIRENLER AYLQYNKCSE ILSKAKFFFP EDEQLLVSEA NLREVGAQLK
 601 QVQGKCQERA QKFAIFEKHI QEQKSLIKEQ VRSFDLAGVG FLKSELLSIA CNLYIKAVVK
 661 ESIPVDVPCM QLYYSYYEDN EAVVRNRLLN MTERYQNFKR SLNSIQFNGD VLLRDPVYQP
 721 EGHETRLKER ELQETTLSCK KLKVAQDRLS ELESRLSRR
```

(54) Hypothetical (CPn0794)

One example of a hypothetical protein is set forth as SEQ ID NO: 54 below. GenBank Accession No. GI:4377105; AAD18932.1. Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 54; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 54, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 54. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 54. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 54. Other fragments omit one or more

domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 54

```
  1 MSLYQKWWNS QLKKSLCYST VAALIFMIPS QESFADSLID LNLGLDPSVE CLSGDGAFSV
 61 GYFTKAGSTP VEYQPFKYDV SKKTFTILSV ETANQSGYAY GISYDGTITV GTCSLGAGKY
121 NGAKWSADGT LTPLTGITGG TSHTEARAIS KDTQVIEGFS YDASGQPKAV QWASGATTVT
181 QLADISGGSR SSYAYAISDD GTIIVGSMES TITRKTTAVK WVNNVPTYLG TLGGDASTGL
241 YISGDGTVIV GAANTATVTN GNQESHAYMY KDNQMKD
```

(55) Hypothetical (CPn0796)

One example of a hypothetical protein is set forth as SEQ ID NO: 55 below. GenBank Accession No. GI:4377107; AAD18934.1. Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 55; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 55, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 55. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 55. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 55. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). Cpn0796 may be secreted from *C. pneumoniae* and is localized in the membrane of Chlamydia in young inclusions whereas an N-terminal part of Cpn0796 is secreted into the host cell cytoplasm at later times. Cpn0796 was proposed to be an autotransporter and it is the first example of secretion into the host cell cytoplasm of a proposed Chlamydia autotrasporter. Te finding in the host cell cytoplasm of Cpn0796 suggests that an unknown transport mechanism exists for translocation over the inclusion membrane (Vandahl, "Proteome analysis of Chlamydia pneumoniae – proteins at the Chlamydia-host cell Interface," Abstract of PhD Dissertation, Dan Med Bull 2004: 51:306).

SEQ ID No 55

```
  1 MQPCLNMSIV RNSALPLPCL SRSETFKKVR SHMKFMKVLT PWIYRKDLWV TAFLLTAIPG
 61 SFAHTLVDIA GEPRHAAQAT GVSGDGKIVI GMKVPDDPFA ITVGFQYIDG HLQPLEAVRP
121 QCSVYPNGIT PDGTVIVGTN YAIGMGSVAV KWVNGKVSEL PMLPDTLDSV ASAVSADGRV
181 IGGNRNINLG ASVAVKWEDD VITQLPSLPD AMNACVNGIS SDGSIIVGTM VDVSWRNTAV
241 QWIGDQLSVI GTLGGTTSVA SAISTDGTVI VGGSENADSQ THAYAYKNGV MSDIGTLGGF
301 YSLAHAVSSD GSVIVGVSTN SEHRYHAFQY ADGQMVDLGT LGGPESYAQG VSGDGKVIVG
361 RAQVPSGDWH AFLCPFQAPS PAPVHGGSTV VTSQNPRGMV DINATYSSLK NSQQQLQRLL
421 IQHSAKVESV SSGAPSFTSV KGAISKQSPA VQNDVQKGTF LSYRSQVHGN VQNQQLLTGA
481 FMDWKLASAP KCGFKVALHY GSQDALVERA ALPYTEQGLG SSVLSGFGGQ VQGRYDFNLG
541 ETVVLQPFMG IQVLHLSREG YSEKNVRFPV SYDSVAYSAA TSFMGAHVFA SLSPKMSTAA
601 TLGVERDLNS HIDEFKGSVS AMGNFVLENS TVSVLRPFAS LAMYYDVRQQ QLVTLSVVMN
661 QQPLTGTLSL VSQSSYNLSF
```

One preferred protein for use with the invention comprises an N-terminal peptide of Cpn0796 that may be secreted to be exposed on the bacterial cell surface and can also become detached via a proteolytic event. In one embodiment, the N-terminal peptide of Cpn0796 may form a beta-propeller structural conformation. One example of the

N-terminal peptide of Cpn0796 is set forth as SEQ ID NO: 86 below. The N-terminal peptide of Cpn0796 for use with the invention may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 86; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 86, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 86. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 86. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 86.

SEQ ID NO: 86

HTLVDIAGEPRHAAQATGVSGDGKIVIGMKVPDDPFAITVGFQYIDGHLQPLEAVRPQCSVYPNG
ITPDGTVIVGTNYAIGMGSVAVKWVNGKVSELPMLPDTLDSVASAVSADGRVIGGNRNINLGASV
AVKWEDDVITQLPSLPDAMNACVNGISSDGSIIVGTMVDVSWRNTAVQWIGDQLSVIGTLGGTTS
VASAISTDGTVIVGGSENADSQTHAYAYKNGVMSDIGTLGGFYSLAHAVSSDGSVIVGVSTNSEH
RYHAFQYADGQMVDLGTLGGPESYAQGVSGDGKVIVGRAQVPSGDWHAFLC

(56) Hypothetical (CPn0797)

One example of a hypothetical protein is set forth as SEQ ID NO: 56 below. GenBank Accession No. GI:4377108; AAD18935.1 Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 56; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 56, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 56. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 56. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 56. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 56

```
  1 MSKKIKVLGH LTLCTLFRGV LCAAALSNIG YASTSQESPY QKSIEDWKGY TFTDLELLSK
 61 EGWSEAHAVS GNGSRIVGAS GAGQGSVTAV IWESHLIKHL GTLGGEASSA EGISKDGEVV
121 VGWSDTREGY THAFVFDGRD MKDLGTLGAT YSVARGVSGD GSIIVGVSAT ARGEDYGWQV
181 GVKWEKGKIK QLKLLPQGLW SEANAISEDG TVIVGRGEIS RNHIVAVKWN KNAVYSLGTL
241 GGSVASAEAI SANGKVIVGW STTNNGETHA FMHKDETMHD LGTLGGGFSV ATGVSADGRA
301 IVGFSAVKTG EIHAFYYAEG EMEDLTTLGG EEARVFDISS EGNDIIGSIK TDAGAERAYL
361 FHIHK
```

(76) Oligopeptide Binding Protein Oppa-2 Lipoprotein (CPn0196)

One example of an oligopeptide binding protein is set forth as SEQ ID NO[s]: 127 and 128 in WO 02/02606. {GenBank accession number GI:4376467; AAD18349.1

'CPn0196'; SEQ ID NO: 76 below}. Preferred oligopeptide binding proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 76; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 76, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO 76. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 76. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 76. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 76

```
   1 mlrffavfis tlwlitsgcs psqsskgifv vnmkemprsl dpgktrliad qtlmrhlyeg
  61 lveehsqnge ikpalaesyt isedgtrytf kiknilwsng dpltaqdfvs swkeilkeda
 121 ssvylyaflp iknaraifdd tespenlgvr aldkrhleiq letpcahflh fltlpiffpv
 181 hetlrnysts feempitcga frpvslekgl rlhleknpmy hnksrvklhk iivqfisnan
 241 taailfkhkk ldwqgppwge pippeisasl hqddqlfslp gasttwllfn iqkkpwnnak
 301 lrkalslaid kdmltkvvyq glaeptdhil hprlypgtyp erkrqneril eaqqlfeeal
 361 delqmtredl eketltfstf sfsygricqm lreqwkkvlk ftipivgqef ftiqknfleg
 421 nysltvnqwt aafidpmsyl mifanpggis pyhlqdshfq tllikitqeh kkhlrnqlii
 481 ealdylehch ileplchpnl rialnknikn fnlfvrrtsd frfiekl
```

Seventh Antigen Group

The immunogenicity of other *Chlamydia pneumoniae* antigens may be improved by combination with two or more *Chlamydia pneumoniae* antigens from either the first antigen group or the second antigen group or the third antigen group or the fourth antigen group or the fifth antigen group or the sixth antigen group. Such other *Chlamydia pneumoniae* antigens include a seventh antigen group consisting one or more hypothetical proteins (ie proteins which, for example, have no known cellular location and/or function. These antigens are referred to herein as the "seventh antigen group". Each of the *Chlamydia pneumoniae* antigens of the seventh antigen group is described in more detail below.

(57) Hypothetical (CPn0331)

One example of a hypothetical protein is set forth as SEQ ID NO: 57 below. GenBank Accession No. GI:4376609; AAD18480.1. Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 57; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 57, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 57. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 57. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 57. Other fragments omit one or more

domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 57

```
  1 MAVSGGGGVQ PSSDPGKWNP ALQGEQAEGP SPLKESIFSE TKQASSAAKQ ESLVRSGSTG
 61 MYATESQINK AKYRKAQDRS STSPKSKLKG TFSKMRASVQ GFMSGFGSRA SRVSAKRASD
121 SGEGTSLLPT EMDVALKKGN RISPEMQGFF LDASGMGGSS SDISQLSLEA LKSSAFSGAR
181 SLSLSSSESS SVASFGSFQK AIEPMSEEKV NAWTVARLGG EMVSSLLDPN VETSSLVRRA
241 MATGNEGMID LSDLGQEEVS TAMTSPRAVE GKVKVSSSDS PEANPTGIPN SNTLERAEKE
301 AEKQESREQL SEDQMMLARA MAGLLTGAAP QEVLSNSVWS GPSTVFPPPK FSGTLPTQRS
361 GDKSKHKSPG IEKSTNHTNF SPLREGTVKS AEVKSLPHPE SMYRFPKDSI VSREEPEAVV
421 KESTAFKNPE NSSQNFLPIA VESVFPKESG TGGALGSDAV SSSYHFLAQR GVSLLAPLPR
481 ATDDYKEKLE AHKGPGGPPD PLIYQYRNVA VEPPIVLRSP QPFSGSSRLS VQGKPEAASV
541 HDDGGGGNSG GFSGDQRRGS SGQKASRQEK KGKKLSTDI
```

(58) Hypothetical (CPn0234)

One example of a hypothetical protein is set forth as SEQ ID NO: 58 below. GenBank Accession No. gi|4376508|gb| AAD18387.1 Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 58; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 21, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 58. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 58. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 58. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 58

```
  1 MLQSCKKALL SIVVSILAFH PIPGMGVEAK SGFLGKVKGW FSKKEIQEEA RILPVKDSLS
 61 WKRYDYTSSS GFSVEFPGEP DHSGQIVEVP QSEITIRYDT YVTETHPDNT VYVVSVWEYP
121 EKVDISRPEL NLQEGFSGMM QALPESQVLF MQARQIQGHK ALEFWIVCED VYFRGMLISV
181 NHTLYQVFMV YKNKNPQALD KEYEAFSQSF KITKIREPRT IPSSVKKKVS L
```

(59) Hypothetical (CPn0572)

One example of a hypothetical protein is set forth as SEQ ID NO: 59 below. Genbank Accession No. gi|4376866|gb|; AAD18712.1. Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 59; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 59, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 59. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 59. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 59. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 59

```
   1 MAAPINQPST TTQITQTGQT TTTTTVGSLG EHSVTTTGSG AAAQTSQTVT LIADHEMQEI
  61 ASQDGSAVSF SAEHSFSTLP PETGSVGATA QSAQSAGLFS LSGRTQRRDS EISSSSDGSS
 121 ISRTSSNASS GETSRAESSP DLGDLDSLSG SERAEGAEGP EGPGGLPEST IPHYDPTDKA
 181 SILNFLKNPA VQQKMQTKGG HFVYVDEARS SFIFVRNGDW STAESIKVSN AKTKENITKP
 241 ADLEMCIAKF CVGYETIHSD WTGRVKPTME ERSGATGNYN HLMLSMKFKT AVVYGPWNAK
 301 ESSSGYTPSA WRRGAKVETG PIWDDVGGLK GINWKTTPAP DFSFINETPG GGAHSTSHTG
 361 PGTPVGATVV PNVNVNLGGI KVDLGGINLG GITTNVTTEE GGGTNITSTK STSTDDKVSI
 421 TSTGSQSTIE EDTIQFDDPG QGEDDNAIPG TNTPPPPGPP PNLSSSRLLT ISNASLNQVL
 481 QNVRQHLNTA YDSNGNSVSD LNQDLGQVVK NSENGVNFPT VILPKTTGDT DPSGQATGGV
 541 TEGGGHIRNI IQRNTQSTGQ SEGATPTPQP TIAKIVTSLR KANVSSSSVL PQPQVATTIT
 601 PQARTASTST TSIGTGTEST STTSTGTGTG SVSTQSTGVG TPTTTTRSTG TSATTTTSSA
 661 STQTPQAPLP SGTRHVATIS LVRNAAGRSI VLQQGGRSQS FPIPPSGTGT QNMGAQLWAA
 721 ASQVASTLGQ VVNQAATAGS QPSSRRSSPT SPRRK
```

Eighth Antigen Group

The immunogenicity of other *Chlamydia pneumoniae* antigens may be improved by combination with two or more *Chlamydia pneumoniae* antigens from either the first antigen group or the second antigen group of the third antigen group or the fourth antigen group or the fifth or the sixth antigen group or the seventh antigen group. Such other *Chlamydia pneumoniae* antigens include an eigth antigen group consisting one or more FACS positive CPn antigens. These antigens are referred to herein as the "eight antigen group". Each of the *Chlamydia pneumoniae* antigens of the eight antigen group is described in more detail below.

(60) Low Calcium Response Protein H (CPn0811)

One example of a Low Calcium Response Protein H is set forth as SEQ ID NO: 60 below. Genbank Accession No. GI:4377123; AAD18949.1. Preferred low calcium response proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 60; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 60, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These low calcium response proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 60. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 60. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 60. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 60

```
   1 mskpsprnan qpqkpsasfn kktrsrlael aaqkkakadd leqvhpvpte eeikkalgni
  61 feglsngldl qqilglsdyl leeiytvayt fysqgkynea vglfqllaaa qpqnykymlg
 121 lsscyhqlhl yneaafgffl afdaqpdnpi ppyyiadsll klqqpeesnn fldvtmdicg
 181 nnpefkilke rcqimkqsie kqmagetkka ptkkpagksk tttnkksgkk r
```

(61) Yop Proteins Translocation Protein T (CPn0823)

One example of a Yop Proteins Translocation Protein T is set forth as SEQ ID NO: 61 below. Genbank Accession No. GI:4377135; AAD18960.1. Preferred Yop proteins

for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 61; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 61, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Yop proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 61. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 61. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 61. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 61

```
  1 mgislpelfs nlgsayldyi fqhppayvws vfllllarll pifavapflg aklfpspiki
 61 gislswlaii fpkvladtqi tnymdnnlfy vllvkemiig ivigfvlafp fyaaqsagsf
121 itnqqgiqgl egatslisie qtsphgilyh yfvtiifwlv gghrivisll lqtlevipih
181 sffpaemmsl sapiwitmik mcqlclvmti qlsapaalam lmsdlflgii nrmapqvqvi
241 yllsalkafm gllfltlaww fiikqidyft lawfkevpim llgsnpqvl
```

(62) Yop Proteins Translocation Protein J

One example of a Yop Proteins Translocation Protein J is set forth as SEQ ID NO: 62 below Genbank Accession No. GI:4377140; AAD18965.1. Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 62; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 62, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 62. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 62. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 62. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 62

```
  1 mvrrsisfcl fflmtllcct scnsrslivh glpgreanei vvllvskgva aqklpqaaaa
 61 tagaateqmw diavpsaqit ealailnqag lprmkgtsll dlfakqglvp selqekiryq
121 eglseqmast irkmdgvvda svqisftten ednlpltasv yikhrgvldn pnsimvskik
181 rliasavpgl vpenvsvvsd raaysditin gpwglteeid yvsvwgiila kssltkfrli
241 fyvlililfv iscgllwviw kthtlimtmg gtkgffnptp ytknaleakk aegaaadkek
301 kedadsqges knaetsdkds sdkdapegsn eiega
```

(63) OmpA (CPn0695)

One example of an OmPA encoded (MOMP) protein is set forth as SEQ ID NO: 63 below Genbank Accession No. GI:4376998; AAD18834.1. Preferred OmpA proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 63; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 63, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These OmpA proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 63. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 63. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 63. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 63

```
  1 mkkllksall saafagsvgs lqalpvgnps dpsllidgti wegaagdpcd pcatwcdais
 61 lragfygdyv fdrilkvdap ktfsmgakpt gsaaanytta vdrpnpaynk hlhdaewftn
121 agfialniwd rfdvfctlga sngyirgnst afnlvglfgv kgttvnanel pnvslsngvv
181 elytdtsfsw svgargalwe cgcatlgaef qyaqskpkve elnvicnvsq fsvnkpkgyk
241 gvafplptda gvatatgtks atinyhewqv gaslsyrlns lvpyigvqws ratfdadnir
301 iaqpklptav lnltawnpsl lgnatalstt dsfsdfmqiv scqinkfksr kacgvtvgat
361 lvdadkwslt aearlinera ahvsgqfrf
```

(64) Hypothetical (CPn0210)

One example of a Hypothetical Protein is set forth as SEQ ID NO: 64 below Genbank Accession No. GI:4376482; AAD18363.1. Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 64; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 64, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 64. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 64. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 64. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 64

```
  1 mlvelealkr efahlkdqkp tsdqeitsly qcldhlefvl lglgqdkflk atededvlfe
 61 sqkaidawna lltkardvlg lgdigaiyqt ieflgaylsk vnrrafcias eihflktair
121 dlnayylldf rwplckieef vdwgndcvei akrklctfek etkelnesll reehamekcs
181 iqdlqrklsd iiielhdvsl fcfsktpsqe eyqkdclyqs rlrylllye ytllcktstd
241 fqeqarakee firekfslle lekgikqtke lefaiakskl ergclvmrky eaaakhslds
301 mfeeetvksp rkdte
```

(65) Low Calcium Response Locus Protein H (CPn1021)

One example of a Low Calcium Response Protein H is set forth as SEQ ID NO: 65 below Genbank Accession No. GI:4377352; AAD19158.1. Preferred low calcium response proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%,

93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 65; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 65, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These low calcium response proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 65. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 65 Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 65. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 65

```
  1 mshlnyllek iaasskedfp fpddlesyle gyvpdknial dtyqkifkis sedlekvyke
 61 gyhayldkdy aksitvfrwl vffnpfvskf wfslgaslhm seqysqalha ygvtavlrdk
121 dpyphyyayi cytltnehee aekalemawv raqhkplyne lkeeildirk hk
```

Ninth Antigen Group

The immunogenicity of other *Chlamydia pneumoniae* antigens may be improved by combination with two or more *Chlamydia pneumoniae* antigens from either the first antigen group or the second antigen group or the third antigen group or the fourth antigen group or the fifth antigen group or the sixth antigen group or the seventh antigen group or the eight antigen group. Such other *Chlamydia pneumoniae* antigens include a ninth antigen group. These antigens are referred to herein as the "ninth antigen group". Each of the *Chlamydia pneumoniae* antigens of the ninth antigen group is described in more detail below.

(66) Low Calcium Response Protein D (CPn0323)

One example of a Low Calcium Response Protein D is set forth as SEQ ID NO: 66 below Genbank Accession No. GI:4376601; AAD18472.1. Preferred low calcium response proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 66; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 66, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These low calcium response proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 66. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 66. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 66. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 66

```
  1 mnkllnfvsr tlggdtalnm inkssdlila lwmmgvvlmi iiplpppivd lmitinlsis
 61 vfllmvalyi psalqlsvfp slllittmfr lginisssrq illkayaghv iqafgdfvvg
121 gnyvvgfiif liitiiqfiv vtkgaervae vaarfrldam pgkqmaidad lragmidatq
181 ardkraqiqk eselygamdg amkfikgdvi agivislini vggltigvam hgmdlaqaah
241 vytllsigdg lvsqipslli altagivttr vssdkntnlg keistqlvke pralllagaa
```

```
301 tlgvgffkgf plwsfsilal ifvalgilll tkksaagkkg ggsgasttvg aagdgaatvg
361 dnpddysltl pvilelgkdl skliqhktks gqsfvddmip kmrqalyqdi girypgihvr
421 tdspslegyd ymillnevpy vrgkipphhv ltnevednls rynlpfityk naaglpsawv
481 sedakailek aaikywtple viilhlsyff hkssqeflgi qevrsmiefm ersfpdlvke
541 vtrliplqkl teifkrlvqe qisikdlrti leslsewaqt ekdtvlltey vrsslklyis
601 fkfsqggqsai svylldpeie emirgaikqt sagsylaldp dsvnlilksm rntitptpag
661 gqppvlltai dvrryvrkli etefpdiavi syqeilpeir iqplgriqif
```

(67) CHLPS 43kDa Protein Homolog-1 (CPn0062)

One example of a CHLPS 43kDa Protein Homolog-1 is set forth as SEQ ID NO: 67 below Genbank Accession No. GI:4376318; AAD18215.1. Preferred CHLPS proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 67; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 67, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These CHLPS proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 67. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 67. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 67. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 67

```
  1 mmsskrtski avlsilltft hsigfanans svglgtvyit sevvkkpqkg serkqakkep
 61 rarkgylvps srtlsaraqk mknssrkess ggcneisans tprsvklrrn kraeqkaakq
121 gfsafsnltl ksllpklpsk qktsiherek atsrfvnesq lssarkryct pssaapslfl
181 eteivrapve rtkelqdnei hipvvqvqtn pkeqntkttk qlasqasiqq segteqslre
241 laqgaslpvl vrsnpevsvq rqkeellkel vaerrqckrk svrqalears ltkkvarggs
301 vtstlrydpe kaaeiksrrn ckvspeareq kyssckrdar angkqdkttp sedasqeeqq
361 tgaglvrktp ksqvasnaqn fyrnskntni dsyltanqys csseetdwpc sscvskrrth
421 nsisvctmvv tviamivgal iianatesqt tsdptpptpt p
```

(68) Hypothetical (CPn0169)

One example of a CHLPS 43kDa Protein Homolog-1 is set forth as SEQ ID NO: 68 below Genbank Accession No. GI:4376437; AAD18322.1. Preferred CHLPS proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 68; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 68, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These CHLPS proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 68. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 68. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 68. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 68

```
   1 mknvgsecsq plvmelntqp lrnlcesrlv kitsfviall alvggitlta lagagilsfl
  61 pwlvlgivlv vlcalfllfs ykfcpikelg vvyntdsqih qwfqkqrnkd lekatenpel
 121 fgenraednn rsarsqvket lrdcdgnvlk kiyernldvl lfmnwvpktm ddvdpvseds
 181 irtvisçykl ikackpefrs lisellramq sglgllsrcs ryqeraktvs hkdaplfcpt
 241 hsyyrdgylt plragpryii nrai
```

(69) PmpD family (CPn0963)

One example of a PmpD protein is set forth as SEQ ID NO: 69 below Genbank Accession No. GI:4377287; AAD19099.1. Preferred PmpD proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 69; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 69, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PmpD proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 69. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 69. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 69. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 69

```
   1 mvakktvrsy rssfshsviv ailsagiafe ahslhsseld lgvfnkqfee hsahveeaqt
  61 svlkgsdpvn psqkesekvl ytqvpltqgs sgesldlada nflehfqhlf eettvfgidq
 121 klvwsdldtr nfsqptqepd tsnavsekis sdtkenrkdl etedpskksg lkevssdlpk
 181 spetavaais edleisenis ardplqglaf fykntssqsi sekdssfqgi ifsgsgansg
 241 lgfenlkapk sgaavysdrd ivfenlvkgl sfiscesled gsaagvnivv thcgdvtltd
 301 catgldleal rlvkdfsrgg avftarnhev qnnlaggils vvgnkgaivv eknsaeksng
 361 gafacgsfvy snnentalwk enqalsggai ssasdidiqg ncsaiefsgn qslialgehi
 421 gltdfvggga laaqgtltlr nnavvqcvkn tskthggail agtvdlneti sevafkqnta
 481 altggalsan dkviiannfg eilfeqnevr nhggaiycgc rsnpkleqkd sgeniniign
 541 sgaitflknk asvlevmtqa edyagggalw ghnvlldsns gniqfignig gstfwigeyv
 601 gggailstdr vtisnnsgdv vfkgnkgqcl aqkyvapqet apvesdasst nkdekslnac
 661 shgdhyppkt veeevppsll eehpvvsstd irgggailaq hifitdntgn lrfsgnlggg
 721 eesstvgdla ivgggallst nevnvcsnqn vvfsdnvtsn gcdsggaila kkvdisanhs
 781 vefvsngsgk fggavcalne svnitdngsa vsfsknrtrl ggagvaapqg svticgnqgn
 841 iafkenfvfg senqrsggga iianssvniq dnagdilfvs nstgsyggai fvgslvaseg
 901 snprtltitg nsgdilfakn stqtaaslse kdsfgggaiy tqnlkivkna gnvsfygnra
 961 psgagvqiad ggtvcleafg gdilfegnin fdgsfnaihl cgndskivel savqdkniif
1021 qdaityeent irglpdkdvs plsapslifn skpqddsaqh hegtirfsrg vskipqiaai
1081 qegtlalsqn aelwlaglkq etgssivlsa gsilrifdsq vdssaplpte nkeetlvsag
1141 vqinmssptp nkdkavdtpv ladiisitvd lssfvpeqdg tlplppeiii pkgtklhsna
1201 idlkiidptn vgyenhalls shkdiplisl ktaegmtgtp tadaslsnik idvslpsitp
1261 atyghtgvws eskmedgrlv vgwqptgykl npekqgalvl nnlwshytdl ralkqeifah
1321 htiaqrmeld fstnvwgsgl gvvedcqnig efdgfkhhlt gyalgldtql vedfliggcf
1381 sqffgktesq sykakndvks ymgaayagil agpwlikgaf vygninndlt tdygtlgist
1441 gswigkgfia gtsidyryiv nprrfisaiv stvvpfveae yvridlpeis eqgkevrtfq
1501 ktrfenvaip fgfalehays rgsraevnsv qlayvfdvyr kgpvslitlk daayswksyg
1561 vdipckawka rlsnntewns ylstylafny ewredliayd fnggiriif
```

Tenth Antigen Group

The immunogenicity of other *Chlamydia pneumoniae* antigens may be improved by combination with two or more *Chlamydia pneumoniae* antigens from either the first

antigen group or the second antigen group or the third antigen group or the fourth antigen group or the fifth antigen group or the sixth antigen group or the seventh antigen group or the eight antigen group or the ninth antigen group. Such other *Chlamydia pneumoniae* antigens include a tenth antigen group. Each of the *Chlamydia pneumoniae* antigens of the tenth antigen group is described in more detail below.

(70) OmpH-like outer membrane protein (CPn0301)

One example of 'OmpH-like' protein is disclosed as SEQ ID NO[s]: 77 & 78 in WO 02/02606. {GenBank accession number: gi|4376577|gb|AAD18450.1| 'CPn0301'; SEQ ID NO: 70 below and SEQ ID No 4 above}. Preferred OmpH-like proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 4; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 3, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These OmpH-like proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 4. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 4. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more; preferably 19 or more, to remove the signal peptide) from the N-terminus of SEQ ID NO: 4. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 70

```
   1  MKKLLFSTFL LVLGSTSAAH ANLGYVNLKR CLEESDLGKK ETEELEAMKQ
  51  QFVKNAEKIE EELTSIYNKL QDEDYMESLS DSASEELRKK FEDLSGEYNA
 101  YQSQYYQSIN QSNVKRIQKL IQEVKIAAES VRSKEKLEAI LNEEAVLAIA
 151  PGTDKTTEII AILNESFKKQ N*
```

(71) L7/L12 Ribosomal Protein (CPn0080)

One example of an L7/L12 Ribosomal protein is set forth as SEQ ID No 71 below{GenBank accession number: GI:4376338; AAD18233.1}. 'CPn0080'; SEQ ID NO: 71 below. Preferred L7/L12 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 71; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 71, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These L7/L12 ribosomal proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 71. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 71. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more; preferably 19 or more, to remove the signal peptide) from the N-terminus of SEQ ID NO: 71. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 71

```
   1 mttesletlv eklsnltvle lsqlkkllee kwdvtasapv vavaagggge apvaaeptef
  61 avtledvpad kkigvlkvvr evtglalkea kemteglpkt vkektsksda edtvkklqda
121gakasfkgl
```

(72) AtoS two-component regulatory system sensor histidine kinase protein (CPn0584)

One example of 'AtoS' protein is disclosed as SEQ ID NO[s]: 105 & 106 in WO 02/02606. {GenBank accession number: gi|4376878|gb|AAD18723.1| 'CPn0584'; SEQ ID NO: 72 below and SEQ ID No 9 above}. Preferred AtoS proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 72; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 72, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These AtoS proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 72. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 72. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 72. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 72

```
1    MNVPDSKNLH PPAYELLEIK ARITQSYKEA SAILTAIPDG ILLLSETGHF
51   LICNSQAREI LGIDENLEIL NRSFTDVLPD TCLGFSIQEA LESLKVPKTL
101  RLSLCKESKE KEVELFIRKN EISGYLFIQI RDRSDYKQLE NAIERYKNIA
151  ELGKMTATLA HEIRNPLSGI VGFASILKKE ISSPRHQRML SSIISGTRSL
201  NNLVSSMLEY TKSQPLNLKI INLQDFFSSL IPLLSVSFPN CKFVREGAQP
251  LFRSIDPDRM NSVVWNLVKN AVETGNSPIT LTLHTSGDIS VTNPGTIPSE
301  IMDKLFTPFF TTKREGNGLG LAEAQKIIRL HGGDIQLKTS DSAVSFFIII
351  PELLAALPKE RAAS*
```

(73) OmcA 9kDa-cysteine-rich lipoprotein(CPn0558)

One example of 'OmcA' protein is disclosed as SEQ ID NO[s]: 9 & 10 in WO 02/02606. {GenBank accession number: gi|4376850|gb|AAD18698.1| 'CPn0558', 'OmcA', 'Omp3'; SEQ ID NO: 73 below and SEQ ID No 10 above}. Preferred OmcA proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 73; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 73, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These OmcA proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 73. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 73. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more; preferably 18 or more to remove the signal peptide) from the N-terminus of SEQ ID NO: 73. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a

transmembrane domain, or of an extracellular domain). The protein may be lipidated (*e.g.* by a *N*-acyl diglyceride), and may thus have a N-terminal cysteine.

SEQ ID No 73

```
  1 MKKAVLIAAM FCGVVSLSSC CRIVDCCFED PCAPSSCNPC EVIRKKERSC
 51 GGNACGSYVP SCSNPCGSTE CNSQSPQVKG CTSPDGRCKQ *
```

(74) Hypothetical (CPn0331)

One example of a hypothetical protein is set forth as SEQ ID NO: 74 below and SEQ ID No 57 above. Genbank Accession No. GI:4376609; AAD18480.1. Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 74; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 74, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 74. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 74. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 74. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO 74

```
   1 mavsggggvq pssdpgkwnp alqgeqaegp splkesifse tkqassaakq eslvrsgstg
  61 myatesqink akyrkaqdrs stspksklkg tfskmrasvq gfmsgfgsra srvsakrasd
 121 sgegtsllpt emdvalkkgn rispemqgff ldasgmggss sdisqlslea lkssafsgar
 181 slslsssess svasfgsfqk aiepmseekv nawtvarlgg emvsslldpn vetsslvrra
 241 matgnegmid lsdlgqeevs tamtsprave gkvkvsssds peanptgipn sntleraeke
 301 aekqesreql sedqmmlara maglltgaap qevlsnsvws gpstvfpppk fsgtlptqrs
 361 gdkskhkspg iekstnhtnf splregtvks aevkslphpe smyrfpkdsi vsreepeavv
 421 kestafknpe nssqnflpia vesvfpkesg tggalgsdav sssyhflaqr gvsllaplpr
 481 atddykekle ahkgpggppd pliyqyrnva veppivlrsp qpfsgssrls vqgkpeaasv
 541 hddggggnsg gfsgdqrrgs sgqkasrqek kgkklstdi
```

(75) PmpD family (CPn0963)

One example of a PmpD protein is set forth as SEQ ID NO: 75 below Genbank Accession No. GI:4377287; AAD19099.1. Preferred PmpD proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 75; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 75, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 75. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 75. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 75. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 75

```
   1 mvakktvrsy rssfshsviv ailsagiafe ahslhsseld lgvfnkqfee hsahveeaqt
  61 svlkgsdpvn psqkesekvl ytqvpltqgs sgesldlada nflehfqhlf eettvfgidq
 121 klvwsdldtr nfsqptqepd tsnavsekis sdtkenrkdl etedpskksg lkevssdlpk
 181 spetavaais edleisenis ardplqglaf fykntssqsi sekdssfqgi ifsgsgansg
 241 lgfenlkapk sgaavysdrd ivfenlvkgl sfiscesled gsaagvnivv thcgdvtltd
 301 catgldleal rlvkdfsrgg avftarnhev qnnlaggils vvgnkgaivv eknsaeksng
 361 gafacgsfvy snnentalwk enqalsggai ssasdidiqg ncsaiefsgn qslialgehi
 421 gltdfvggga laaqgtltlr nnavvqcvkn tskthggail agtvdlneti sevafkqnta
 481 altggalsan dkviiannfg eilfeqnevr nhggaiycgc rsnpkleqkd sgeniniign
 541 sgaitflknk asvlevmtqa edyagggalw ghnvlldsns gniqfignig gstfwigeyv
 601 gggailstdr vtisnnsgdv vfkgnkgqcl aqkyvapqet apvesdasst nkdekslnac
 661 shgdhyppkt veeevppsll eehpvvsstd irgggailaq hifitdntgn lrfsgnlggg
 721 eesstvgdla ivgggallst nevnvcsnqn vvfsdnvtsn gcdsggaila kkvdisanhs
 781 vefvsngsgk fggavcalne svnitdngsa vsfsknrtrl ggagvaapqg svticgnqgn
 841 iafkenfvfg senqrsggga iianssvniq dnagdilfvs nstgsyggai fvgslvaseg
 901 snprtltitg nsgdilfakn stqtaaslse kdsfgggaiy tqnlkivkna gnvsfygnra
 961 psgagvqiad ggtvcleafg gdilfegnin fdgsfnaihl cgndskivel savqdkniif
1021 qdaityeent irglpdkdvs plsapslifn skpqddsaqh hegtirfsrg vskipqiaai
1081 qegtlalsqn aelwlaglkq etgssivlsa gsilrifdsq vdssaplpte nkeetlvsag
1141 vqinmssptp nkdkavdtpv ladiisitvd lssfvpeqdg tlplppeiii pkgtklhsna
1201 idlkiidptn vgyenhalls shkdiplisl ktaegmtgtp tadaslsnik idvslpsitp
1261 atyghtgvws eskmedgrlv vgwqptgykl npekqgalvl nnlwshytdl ralkqeifah
1321 htiaqrmeld fstnvwgsgl gvvedcqnig efdgfkhhlt gyalgldtql vedfliggcf
1381 sqffgktesq sykakndvks ymgaayagil agpwlikgaf vygninndlt tdygtlgist
1441 gswigkgfia gtsidyryiv nprrfisaiv stvvpfveae yvridlpeis eqgkevrtfq
1501 ktrfenvaip fgfalehays rgsraevnsv qlayvfdvyr kgpvslitlk daayswksyg
1561 vdipckawka rlsnntewns ylstylafny ewredliayd fnggiriif
```

(76) Hypothetical (CPn0798)

One example of a hypothetical protein is set forth as SEQ ID NO: 78 below. GenBank Accession No. GI:4377109; AAD18936 Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 78; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 78, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 78. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 78. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 78. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 78

```
   1 mkktccqnyr sigvvfsvvl fvlttqtlfa ghfidigtsg lyswargvsg dgrvvvgyeg
  61 gnafkyvdge kflleglvpr sealvfkasy dgsviigisd qdpscravkw vngalvdlgi
 121 fsegmqsfae gvssdgktiv gclysddtet nfavkwdetg mvvlpnlped rhscawdase
 181 dgsvivgdam gseeiakavy wkdgeqhlls nipgakrssa havskdgsfi vgefiseene
 241 vhafvyhngv ikdigtlggd ysvatgvsrd gkvivghstr tdgeyrafky vdgrmidlgt
 301 lggsasfafg vsddgktivg kfetelgech afiyldd
```

(77) Hypothetical (CPn0799)

One example of a hypothetical protein is set forth as SEQ ID NO: 79 below. GenBank Accession No. GI: 15618708; AAD18937 Preferred hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 79; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 79, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 79. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 79. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 79. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 79

```
  1 maaikqilrs mlsqsslwmv lfslyslsgy cyvitdkped dfhsssavkw dhwgkttlsr
 61 lsnkkasaka vsgtgattvg fikdtwsrty avrwnywgtk elptsswvkk skatgissdg
121 siiagivene lsqsfavtwk nnemyllpst wavqskaygi ssdgsvivgs akdawsrtfa
181 vkwtgheaqv lpvgwavksv ansvsangsi ivgsvqdasg ilyavkwegn tithlgtlgg
241 ysaiakavsn ngkvivgrse tyygevhafc hkngvmsdlg tlggsysaak gvsatgkviv
301 gmsttangkl hafkyvggrm idlgeyswke acanavsidg eiivgvqse
```

Preferably the composition of the invention comprises a combination of CPn antigens selected from the group consisting of: (1) CPn0301 and CPn0080; (2) CPn 0584 and CPn 0558; and (3) CPn 0331 and CPN 0963. Preferably the composition comprises a combination of any one or more of groups (1), (2) and (3).

Even more preferably, the composition of the present invention comprises a combination of CPn antigens selected from the group consisting of (1) CPn0385, CPn0324, CPn 0503, CPn0525 and CPn 0482. Preferably the composition is administered in the presence of alum and/or cPG.

The invention thus includes a composition comprising a combination of *Chlamydia pneumoniae* antigens, said combination selected from the group consisting of two, three, four, five or six *Chlamydia pneumoniae* antigens of the first antigen group and two, three, four, five, or six *Chlamydia pneumoniae* antigens of the second antigen group. Preferably, the combination is selected from the group consisting of three, four, five or six *Chlamydia pneumoniae* antigens from the first antigen group and three, four, five or six *Chlamydia pneumoniae* antigens from the second antigen group. Still more preferably, the combination consists of six *Chlamydia pneumoniae* antigens from the first antigen group and three, four, five or six, *Chlamydia pneumoniae* antigens from the second antigen group.

The invention further includes a composition comprising a combination of *Chlamydia pneumoniae* antigens, said combination selected from the group consisting of two, three, four, five or six, *Chlamydia pneumoniae* antigens of the second antigen group and two, three, four, five, six, seven or eight *Chlamydia pneumoniae* antigens of the third antigen group. Preferably, the combination is selected from the group consisting of three, four, five or six *Chlamydia pneumoniae* antigens from the second antigen

group and three, four, five, six, seven or eight *Chlamydia pneumoniae* from the third antigen group. Still more preferably, the combination consists of six *Chlamydia pneumoniae* antigens from the second antigen group and three, four, five, six, seven or eight *Chlamydia pneumoniae* antigens of the third antigen group.

There is an upper limit to the number of *Chlamydia pneumoniae* antigens which will be in the compositions of the invention. Preferably, the number of *Chlamydia pneumoniae* antigens in a composition of the invention is less than 20, less than 19, less than 18, less than 17, less than 16, less than 15, less than 14, less than 13, less than 12, less than 11, less than 10, less than 9, less than 8, less than 7, less than 6, less than 5, less than 4, or less than 3. Still more preferably, the number of *Chlamydia pneumoniae* antigens in a composition of the invention is less than 6, less than 5, or less than 4. The *Chlamydia pneumoniae* antigens used in the invention are preferably isolated, i.e., separate and discrete, from the whole organism with which the molecule is found in nature or, when the polynucleotide or polypeptide is not found in nature, is sufficiently free of other biological macromolecules so that the polynucleotide or polypeptide can be used for its intended purpose.

In either of the above combinations, preferably the composition comprises one or more *Chlamydia pneumoniae* antigens from the fourth antigen group which includes porB. Or, alternatively, in either of the above combinations, preferably the *Chlamydia pneumoniae* antigens from the fourth antigen group includes one or more members of the pmp3 family.

Other aspects of the present invention are presented in the accompanying claims and in the following description and drawings. These aspects are presented under separate section headings. However, it is to be understood that the teachings under each section are not necessarily limited to that particular section heading.

Before describing the present invention in detail, it is to be understood that this invention is not limited to particularly exemplified molecules or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting. In addition, the practice of the present invention will employ, unless otherwise indicated, conventional methods of virology, microbiology, molecular biology, recombinant DNA techniques and immunology all of which are within the ordinary skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *DNA Cloning: A Practical Approach*, vol. I & II (D. Glover, ed.); *Oligonucleotide Synthesis* (N. Gait, ed., 1984); *A Practical Guide to Molecular Cloning* (1984); and *Fundamental Virology*, 2nd Edition, vol. I & II (B.N. Fields and D.M. Knipe, eds.).

All publications, patents and patent applications cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entirety. It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. All scientific and technical terms used in this application have meanings commonly used in the art unless otherwise specified. As used in this application, the following words or phrases have the meanings specified.

The term "comprising" means "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* means, for example, $x \pm 10\%$.
References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987) Supplement 30. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in Smith & Waterman (1981) *Adv. Appl. Math.* 2: 482-489.

IMMUNE RESPONSE
The mechanism by which the immune system controls disease includes the induction of neutralising antibodies via humoral immunity and the generation of T-cell responses via cellular immunity. As used herein, the term "immune response" against an antigen refers to the development in a host mammalian subject of a humoral and/or a cellular immune response against that antigen.
As used herein, the term "humoral immune response" refers to an immune response mediated by antibody molecules. The antibodies generated by humoral immunity are primarily effective against extracellular infectious agents.

SEQ ID Nos 1-86 in the compositions of the invention may be supplemented or substituted with an antibody that binds to the protein. This antibody may be monoclonal or polyclonal.

As used herein, the term "cell mediated immune (CMI) response" is one mediated by T-lymphocytes and/or other white blood cells. The CMI immune mechanisms are generally more effective against intracellular infections and disease because the CMI mechanisms prime T cells in a way that, when an antigen appears at a later date, memory T cells are activated to result in a CMI response that destroys target cells that have the corresponding antigen or a portion thereof on their cell surfaces, and thereby the infecting pathogen. The CMI response is focused on the destruction of the source of infection mediated by either effector cells that destroy infected cells of the host by direct cell-to-cell contact and/or by the release of molecules, such as cytokines, that possess anti-viral activity. Thus the CMI response, which is characterised by a specific T lymphocyte cellular response, is crucial to produce resistance to diseases caused by cancer, viruses, pathogenic and other intracellular microorganisms.

In one aspect of the present invention, an immunogenic composition is provided comprising a combination of at least one antigen that elicits a *Chlamydia pneumoniae* specific Th1 immune response (such as a cell mediated or cellular immune response) and at least one antigen that elicits a *Chlamydia pneumoniae* specific Th2 response (such as a humoral or antibody response). The immunogenic composition may further comprise a Th1 adjuvant and a Th2 adjuvant.

In one embodiment, the invention provides a composition comprising a combination of *Chlamydia pneumoniae* antigens that elicit at least a *Chlamydia pneumoniae* specific Th1 immune response. As an example, the combination of *Chlamydia pneumoniae* antigens may include at least one antigen associated with reticulate bodies (RBs) of *Chlamydia pneumoniae*, including but not limited to antigens expressed, exposed on or translocated into, through or across on the inclusion membrane, antigens expressed, secreted, released or translocated into the cytosol of host cells, or antigens processed or degraded in the cytosol of host cells and/or expressed, exposed or presented on the surface of the host cell. The compositions of the invention will preferably elicit both a cell mediated immune response as well as a humoral immune response in order to effectively address a *Chlamydia* intracellular infection. This immune response will preferably induce long lasting (eg neutralising) antibodies and a cell mediated immunity that can quickly respond upon exposure to *Chlamydia*.

The invention also comprises an immunogenic composition comprising one or more immunoregulatory agents. Preferably, one or more of the immunoregulatory agents include an adjuvant. The adjuvant may be selected from one or more of the group consisting of a Th1 adjuvant and Th2 adjuvant, further discussed below. The adjuvant may be selected from the group consisting of a mineral salt, such as an aluminium salt and an oligonucleotide containing a CpG motif. Most preferably, the immunogenic composition includes both an aluminium salt and an oligonucleotide containing a CpG motif. Use of the combination of a mineral salt, such as an aluminium salt, and an oligonucleotide containing a CpG motif provide for an enhanced immune response. This improved immune response is wholly unexpected and could not be predicted from the use of either agent alone. The invention therefore includes an oligonucleotide containing a CpG motif, a mineral salt such as an aluminium salt, and an antigen, such as a *Chlamydia pneumoniae* antigen.

T CELLS IMPLICATED IN THE CMI RESPONSE

At least two special types of T cells are required to initiate and/or to enhance CMI and and humoral responses. The antigenic receptors on a particular subset of T cells which express a CD4 co-receptor can be T helper (Th) cells or CD4 T cells (herein after called T helper cells) and they recognise antigenic peptides bound to MHC class II molecules. In contrast, the antigenic receptors on a particular subset of T cells which express a CD8 co-receptor are called Cytotoxic T lymphocytes (CTLs) or CD8+ T cells (hereinafter called CD8+ T cells) and they react with antigens displayed on MHC Class I molecules.

HELPER T CELLS

Helper T cells or CD4+ cells can be further divided into two functionally distinct subsets: Th1 and Th2 which differ in their cytokine and effector function. Th1 and Th2 responses have been shown to be regulated not only in a positive but also in a negative way such that Th1 cellular responses are augmented by Th1 cytokines such as IL-2, IL-12 and IFN-gamma and decreased by Th2 cytokines such as IL-4 and IL-10. In contrast, antibody responses are enhanced by Th2 cytokines such as IL-4 and IL-10 but are downregulated by Th1 cytokines such as IFN-gamma and another cytokine IL-12 that enhances IFN-gamma and is produced by monocytes. Thus, classic Th1 cytokines such as IFN-gamma, IL-2 and IL-12 can be regarded as immune

co-factors that induce an effective inflammatory response. In contrast, the classic Th2 cytokines such as IL-4 and IL-10 can be regarded as cytokines that will suppress a severe inflammatory response.

CD8+ T CELLS

CD8+ T cells may function in more than one way. The best known function of CD8+ T cells is the killing or lysis of target cells bearing peptide antigen in the context of an MHC class I molecule. Hence the reason why these cells are often termed cytotoxic T lymphocytes (CTL). However, another function, perhaps of greater protective relevance in certain infections is the ability of CD8+ T cells to secrete interferon gamma (IFN-gamma). Thus assays of lytic activity and of IFN-gamma release are both of value in measuring CD8+ T cell immune response (eg in an ELISPOT assay as set forth below). In infectious diseases there is evidence to suggest that CD8+ T cells can protect by killing an infectious agent comprising an infectious antigen at the early stages of a disease before any symptoms of disease are produced.

ENHANCED CMI RESPONSE

The present invention concerns methods, processes and compositions capable of enhancing and/or modulating the CMI response in a host subject against a target antigen. As used herein, the term "enhancing" encompasses improvements in all aspects of the CMI response which include but are not limited to a stimulation and/or augmentation and/or potentiation and/or up-regulation of the magnitude and/or duration, and/or quality of the CMI response to an antigen or a nucleotide sequence encoding an antigen of interest. By way of example, the CMI response may be enhanced by either (i) enhancing the activation and/or production and/or proliferation of CD8+ T cells that recognise a target antigen and/or (ii) shifting the CMI response from a Th2 to a Thl type response. This enhancement of the Thl associated responses is of particular value in responding to intracellular infections because, as explained above, the CMI response is enhanced by activated Thl (such as, for example, IFN-gamma inducing) cells.

Such an enhanced immune response may be generally characterized by increased titers of interferon-producing $CD4^+$ and/or $CD8^+$ T lymphocytes, increased antigen-specific CD8+ T cell activity, and a T helper 1-like immune response (Th1) against the antigen of interest (characterized by increased antigen-specific antibody titers of the subclasses typically associated with cellular immunity (such as, for example IgG2a), usually with a concomitant reduction of antibody titers of the subclasses typically associated with humoral immunity (such as, for example IgG1)) instead of a T helper 2-like immune response (Th2).

The enhancement of a CMI response may be determined by a number of well-known assays, such as by lymphoproliferation (lymphocyte activation) assays, CD8+ T cell assays, or by assaying for T-lymphocytes specific for the epitope in a sensitized subject (see, for example, Erickson *et al.* (1993) J. Immunol. 151: 4189-4199; and Doe *et al.* (1994) Eur. J. Immunol. 24: 2369-2376) or CD8+ T cell ELISPOT assays for measuring Interferon gamma production (Miyahara *et al* PNAS(USA) (1998) 95: 3954-3959).

ENHANCED T-CELL RESPONSE

As used herein, the term "enhancing a T -cell response" encompasses improvements in all aspects of the T-cell response which include but are not limited to a stimulation and/or augmentation and/or potentiation and/or up-regulation of the magnitude and/or duration, and/or quality of the T-cell response to an antigen (which may be repeatedly administered) or a nucleotide sequence encoding an antigen. The antigen may be a *Chlamydia* antigen, preferably a *Chlamydia pneumoniae* antigen. By way of example, the T-cell response may be enhanced by either enhancing the activation and/or production and/or distribution and/or proliferation of the induced T-cells and/or longevity of the T-cell response to T-cell inducing/modulating antigen or nucleotide sequence encoding an antigen. The enhancement of the T-cell response in a host subject may be associated with the enhancement and/or modulation of the Th1 immune response in the host subject.

The enhancement of the T-cell response may be determined by a number of well-known assays, such as by lymphoproliferation (lymphocyte activation) assays, CD8+ T-cell cytotoxic cell assays, or by assaying for T-lymphocytes specific for the epitope in a sensitized subject (see, for example, Erickson *et al.* (1993) J. Immunol. 151: 4189-4199; and Doe *et al.* (1994) Eur. J. Immunol. 24: 2369-2376) or CD8+ T-cell ELISPOT assays for measuring Interferon gamma production (Miyahara *et al* PNAS(USA) (1998) 95: 3954-3959).

Activated Th1 cells enhance cellular immunity (including an increase in antigen-specific CTL production) and are therefore of particular value in responding to intracellular infections. Activated Th1 cells may secrete one or more of IL-2, IFN-gamma, and TNF-beta. A Th1 immune response may result in local inflammatory reactions by activating macrophages, NK (natural killer) cells, and CD8 cytotoxic T cells (CTLs). A Th1 immune response may also act to expand the immune response by stimulating growth of B and T cells with IL-12. Th1 stimulated B cells may secrete IgG2a.

Activated Th2 cells enhance antibody production and are therefore of value in responding to extracellular infections. Activated Th2 cells may secrete one or more of IL-4, IL-5, IL-6, and IL-10. A Th2 immune response may result in the production of IgG1, IgE, IgA and memory B cells for future protection.

ANTIGEN

Each disease causing agent or disease state has associated with it an antigen or immunodominant epitope on the antigen which is crucial in immune recognition and ultimate elimination or control of a disease causing agent or disease state in a host. In order to mount a humoral and/or cellular immune response against a particular disease, the host immune system must come in contact with an antigen or an immunodominant epitope on an antigen associated with that disease state.

As used herein, the term "antigen" refers to any agent, generally a macromolecule, which can elicit an immunological response in an individual. The term "antigen" is used interchangeably with the term "immunogen". The immunological response may be of B- and/or T-lymphocytic cells. The term may be used to refer to an individual macromolecule or to a homogeneous or heterogeneous population of antigenic macromolecules. As used herein, "antigen" is used to refer to a protein molecule or

portion thereof which contains one or more antigenic determinants or epitopes. As used herein, the term "antigen" means an immunogenic peptide or protein of interest comprising one or more epitopes capable of inducing a CMI response to an infectious *Chlamydia* pathogen. The antigen can include but is not limited to an auto-antigen, a self-antigen, a cross-reacting antigen, an alloantigen, a tolerogen, an allergen, a hapten, an immunogen or parts thereof as well as any combinations thereof.

EPITOPE

As used herein, the term "epitope" generally refers to the site on an antigen which is recognised by a T-cell receptor and/or an antibody. Preferably it is a short peptide derived from or as part of a protein antigen. However the term is also intended to include peptides with glycopeptides and carbohydrate epitopes. Several different epitopes may be carried by a single antigenic molecule. The term "epitope" also includes modified sequences of amino acids or carbohydrates which stimulate responses which recognise the whole organism. It is advantageous if the selected epitope is an epitope of an infectious agent, such as a *Chlamydia* bacterium, which causes the infectious disease.

SEQ ID Nos 1-86 in the compositions of the invention may be supplemented or substituted with molecules comprising fragments of SEQ ID Nos 1-86. Such fragments may comprise at least n consecutive monomers from the molecules and. depending on the particular sequence. n is either (i) 7 or more for protein molecules (eg. 8 18, 20 or more), preferably such that the fragment comprises an epitope from the sequence. or (ii) 10 or more for nucleic acid molecules (eg 15, 18, 20, 25, 30, 35, 40 or more).

SOURCE OF EPITOPES

The epitope can be generated from knowledge the amino acid and corresponding DNA sequences of the peptide or polypeptide, as well as from the nature of particular amino acids (e.g., size, charge, etc.) and the codon dictionary, without undue experimentation. See, e.g., Ivan Roitt, Essential Immunoloqy, 1988; Kendrew, supra; Janis Kuby, Immunology, 1992 e.g., pp. 79-81. Some guidelines in determining whether a protein will stimulate a response, include: Peptide length—preferably the peptide is about 8 or 9 amino acids long to fit into the MHC class I complex and about 13-25 amino acids long to fit into a class II MHC complex. This length is a minimum for the peptide to bind to the MHC complex. It is preferred for the peptides to be longer than these lengths because cells may cut peptides. The peptide may contain an appropriate anchor motif which will enable it to bind to the various class I or class II molecules with high enough specificity to generate an immune response (See Bocchia, M. *et al*, Specific Binding of Leukemia Oncogene Fusion Protein Pentides to HLA Class I Molecules, Blood 85:2680-2684; Englehard, VH, Structure of peptides associated with class I and class II MHC molecules Ann. Rev. Immunol. 12:181 (1994)). This can be done, without undue experimentation, by comparing the sequence of the protein of interest with published structures of peptides associated with the MHC molecules. Thus, the skilled artisan can ascertain an epitope of interest by comparing the protein sequence with sequences listed in the protein data base.

T CELL EPITOPES

Preferably one or more antigens of the present invention contain one or more T cell epitopes. As used herein, the term "T cell epitope" refers generally to those features

of a peptide structure which are capable of inducing a T cell response. In this regard, it is accepted in the art that T cell epitopes comprise linear peptide determinants that assume extended conformations within the peptide-binding cleft of MHC molecules (Unanue *et al.* (1987) Science 236: 551-557). As used herein, a T cell epitope is generally a peptide having at least about 3-5 amino acid residues, and preferably at least 5-10 or more amino acid residues. However, as used herein, the term "T cell epitope" encompasses any MHC Class I-or MHC Class II restricted peptide. The ability of a particular T cell epitope to stimulate/enhance a CMI response may be determined by a number of well-known assays, such as by lymphoproliferation (lymphocyte activation) assays, CD8+ T-cell cytotoxic cell assays, or by assaying for T-lymphocytes specific for the epitope in a sensitized subject. See, e. g., Erickson *et al.* (1993) J. Immunol. 151: 4189-4199; and Doe *et al.* (1994) Eur. J. Immunol. 24: 2369-2376 or CD8+ T-cell ELISPOT assays for measuring Interferon gamma production (Miyahara *et al* PNAS(USA) (1998) 95: 3954-3959).

CD8+ T-CELL EPITOPES

Preferably the antigens of the present invention comprisse CD8+ T-cell inducing epitopes. A CD8+ T-cell –inducing epitope is an epitope capable of stimulating the formation, or increasing the activity, of specific CD8+ T-cells following its administration to a host subject. The CD8+ T-cell epitopes may be provided in a variety of different forms such as a recombinant string of one or two or more epitopes. CD8+ T-cell epitopes have been identified and can be found in the literature, for many different diseases. It is possible to design epitope strings to generate CD8+ T-cell response against any chosen antigen that contains such CD8+ T-cell epitopes. Advantageously, CD8+ T-cell inducing epitopes may be provided in a string of multiple epitopes which are linked together without intervening sequences so that unnecessary nucleic acid material is avoided.

T HELPER EPITOPES

Preferably the antigens of the present invention comprise helper T lymphocyte epitopes. Various methods are available to identify T helper cell epitopes suitable for use in accordance herewith. For example, the amphipathicity of a peptide sequence is known to effect its ability to function as a T helper cell inducer. A full discussion of T helper cell-inducing epitopes is given in U.S. Patent 5,128,319, incorporated herein by reference.

B CELL EPITOPES

Preferably the antigens of the present invention comprise a mixture of CD8+ T-cell epitopes and B cell epitopes. As used herein, the term "B cell epitope" generally refers to the site on an antigen to which a specific antibody molecule binds. The identification of epitopes which are able to elicit an antibody response is readily accomplished using techniques well known in the art. See, e. g., Geysen *et al.* (1984) Proc. Natl. Acad. Sci. USA 81: 3998-4002 (general method of rapidly synthesizing peptides to determine the location of immunogenic epitopes in a given antigen); U. S. Patent No. 4,708,871 (procedures for identifying and chemically synthesizing epitopes of antigens); and Geysen *et al.*(1986) Molecular Immunology 23: 709-715 (technique for identifying peptides with high affinity for a given antibody).

COMBINATION OF EPITOPES

In a preferred embodiment of the present invention, the antigen or antigen combination comprises a mixture of a CD8+ T-cell –inducing epitopes and a T helper cell-inducing epitopes.

As is well known in the art, T and B cell inducing epitopes are frequently distinct from each other and can comprise different peptide sequences. Therefore certain regions of a protein's peptide chain can possess either T cell or B cell epitopes. Therefore, in addition to the CD8+ T-cell epitopes, it may be preferable to include one or more epitopes recognised by T helper cells, to augment the immune response generated by the CD8+ T-cell epitopes.

The mechanism of enhancing a CD8+ T-cell induced response *in vivo* by T helper cell inducing agents is not completely clear. However, without being bound by theory, it is likely that the enhancing agent, by virtue of its ability to induce T helper cells, will result in increased levels of necessary cytokines that assist in the clonal expansion and dissemination of specific CD8+ T-cells. Regardless of the underlying mechanism, it is envisioned that the use of mixtures of helper T cell and CD8+ T-cell -inducing antigen combinations of the present invention will assist in the enhancement of the CMI response. Particularly suitable T helper cell epitopes are ones which are active in individuals of different HLA types, for example T helper epitopes from tetanus (against which most individuals will already be primed). It may also be useful to include B cell epitopes for stimulating B cell responses and antibody production. Synthetic nucleotide sequences may also be constructed to produce two types of immune responses: T cell only and T cell combined with a B cell response.

IMMUNODOMINANT EPITOPE

When an individual is immunized with an antigen or combination of antigens or nucleotide sequence or combinations of nucleotide sequences encoding multiple epitopes of a target antigen, in many instances the majority of responding T lymphocytes will be specific for one or more linear epitopes from that target antigen and/or a majority of the responding B lymphocytes will be specific for one or more linear or conformational epitopes for the antigen or combination of antigens.. For the purposes of the present invention, then, such epitopes are referred to as "immunodominant epitopes". In an antigen having several immunodominant epitopes, a single epitope may be the most dominant in terms of commanding a specific T or B cell response.

As the Examples show, at least sixteen peptides of the present invention were recognised by IFN-gamma positive CD8+ T cell populations which were actually expanded as a result of bacterial infection.

ADJUVANTS

The compositions of the present invention may be administered in conjunction with other immunoregulatory agents. In particular, the compositions of the present invention may be administered with an adjuvant.

The inclusion of an adjuvant and in particular, a genetic adjuvant may be useful in further enhancing or modulating the CMI response. An adjuvant may enhance the CMI response by enhancing the immunogenicity of a co-administered antigen in an

immunized subject, as well inducing a Th1-like immune response against the co-administered antigen which is beneficial in a vaccine product.

An immune response and particularly a CMI response may be refined, by the addition of adjuvants to combinations of antigens or nucleotide sequences encoding combinations of antigens which lead to particularly effective compositions for eliciting a long lived and sustained enhanced CMI response.

As used herein, the term "adjuvant" refers to any material or composition capable of specifically or non-specifically altering, enhancing, directing, redirecting, potentiating or initiating an antigen-specific immune response.

The term "adjuvant" includes but is not limited to a bacterial ADP-ribosylating exotoxin, a biologically active factor, immunomodulatory molecule, biological response modifier or immunostimulatory molecule such as a cytokine, an interleukin, a chemokine or a ligand or an epitope (such as a helper T cell epitope) and optimally combinations thereof which, when administered with an antigen, antigen composition or nucleotide sequence encoding such antigens enhances or potentiates or modulates the CMI response relative to the CMI response generated upon administration of the antigen or combination of antigens alone. The adjuvant may be any adjuvant known in the art which is appropriate for human or animal use.

Immunomodulatory molecules such as cytokines (TNF-alpha, IL-6, GM-CSF, and IL-2), and co-stimulatory and accessory molecules (B7-1, B7-2) may be used as adjuvants in a variety of combinations. In one embodiment GM-CSF is not administered to subject before, in or after the administration regimen. Simultaneous production of an immunomodulatory molecule and an antigen of interest at the site of expression of the antigen of interest may enhance the generation of specific effectors which may help to enhance the CMI response. The degree of enhancement of the CMI response may be dependent upon the specific immunostimulatory molecules and/or adjuvants used because different immunostimulatory molecules may elicit different mechanisms for enhancing and/or modulating the CMI response. By way of example, the different effector mechanisms/immunomodulatory molecules include but are not limited to augmentation of help signal (IL-2), recruitment of professional APC (GM-CSF), increase in T cell frequency (IL-2), effect on antigen processing pathway and MHC expression (IFN-gamma and TNF-alpha) and diversion of immune response away from the Th1 response and towards a Th2 response (LTB) (see WO 97/02045). Unmethylated CpG containing oligonucleotides (see WO96/02555) are also preferential inducers of a Th1 response and are suitable for use in the present invention.

Without being bound by theory, the inclusion of an adjuvant is advantageous because the adjuvant may help to enhance the CMI response to the expressed antigen by diverting the Th2 response to a Th1 response and/or specific effector associated mechanisms to an expressed epitope with the consequent generation and maintenance of an enhanced CMI response (see, for example, the teachings in WO 97/02045).

The inclusion of an adjuvant with an antigen or nucleotide sequence encoding the antigen is also advantageous because it may result in a lower dose or fewer doses of the antigen/antigenic combination being necessary to achieve the desired CMI response in

the subject to which the antigen or nucleotide sequence encoding the antigen is administered, or it may result in a qualitatively and/or quantitatively different immune response in the subject. The effectiveness of an adjuvant can be determined by administering the adjuvant with the antigen in parallel with the antigen alone to animals and comparing antibody and/or cellular-mediated immunity in the two groups using standard assays such as radioimmunoassay, ELISAs, CD8+ T-cell assays, and the like, all well known in the art. Typically, the adjuvant is a separate moiety from the antigen, although a single molecule (such for example, CTB) can have both adjuvant and antigen properties.

As used herein, the term "genetic adjuvant" refers to an adjuvant encoded by a nucleotide sequence and which, when administered with the antigen enhances the CMI response relative to the CMI response generated upon administration of the antigen alone.

Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. Preferably, the protein is derived from *E. coli* (i.e., E. coli heat labile enterotoxin "LT), cholera ("CT"), or pertussis ("PT").
In one preferred embodiment, the genetic adjuvant is a bacterial ADP-ribosylating exotoxin.

ADP-ribosylating bacterial toxins are a family of related bacterial exotoxins and include diphtheria toxin (DT), pertussis toxin (PT), cholera toxin (CT), the *E. coli* heat-labile toxins (LT1 and LT2), *Pseudomonas* endotoxin A, *Pseudomonas* exotoxin S, *B. cereus* exoenzyme, *B. sphaericus* toxin, *C. botulinum* C2 and C3 toxins, *C. limosum* exoenzyme, as well as toxins from *C. perfringens*, *C. spiriforma* and *C. difficile*, *Staphylococcus aureus* EDIN, and ADP-ribosylating bacterial toxin mutants such as CRM_{197}, a non-toxic diphtheria toxin mutant (see, e.g., Bixler *et al.* (1989) *Adv. Exp. Med. Biol.* 251:175; and Constantino *et al.* (1992) *Vaccine*). Most ADP-ribosylating bacterial toxins are organized as an A:B multimer, wherein the A subunit contains the ADP-ribosyltransferase activity, and the B subunit acts as the binding moiety. Preferred ADP-ribosylating bacterial toxins for use in the compositions of the present invention include cholera toxin and the *E. coli* heat-labile toxins.

Cholera toxin (CT) and the related *E. coli* heat labile enterotoxins (LT) are secretion products of their respective enterotoxic bacterial strains that are potent immunogens and exhibit strong toxicity when administered systemically, orally, or mucosally. Both CT and LT are known to provide adjuvant effects for antigen when administered via the intramuscular or oral routes. These adjuvant effects have been observed at doses below that required for toxicity. The two toxins are extremely similar molecules, and are at least about 70-80% homologous at the amino acid level.

Preferably the genetic adjuvant is cholera toxin (CT), enterotoxigenic *E. Coli* heat-labile toxin (LT), or a derivative, subunit, or fragment of CT or LT which retains adjuvanticity. In an even more preferred embodiment, the genetic adjuvant is LT. In another preferred embodiment, the genetic adjuvant may be CTB or LTB.

Preferably the entertoxin is a non-toxic enterotoxin.
The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in WO 95/17211 and as parenteral adjuvants in WO 98/42375. The toxin or toxoid is

preferably in the form of a holotoxin, comprising both A and B subunits. Preferably, the A subunit contains a detoxifying mutation; preferably the B subunit is not mutated. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63, LT-R72, and LTR192G. The use of ADP-ribosylating toxins and detoxified derivaties thereof, particularly LT-K63 and LT-R72, as adjuvants can be found in the following references each of which is specifically incorporated by reference herein in their entirety (Beignon, *et al.* Infection and Immunity (2002) 70(6):3012 – 3019; Pizza, *et al.*, Vaccine (2001) 19:2534 – 2541; Pizza, *et al.*, Int. J. Med. Microbiol (2000) 290(4-5):455-461; Scharton-Kersten *et al.* Infection and Immunity (2000) 68(9):5306 – 5313; Ryan *et al.* Infection and Immunity (1999) 67(12):6270 – 6280; Partidos *et al.* Immunol. Lett. (1999) 67(3):209 – 216; Peppoloni *et al.* Vaccines (2003) 2(2):285 – 293; and Pine *et al* J. Control Release (2002) 85(1-3):263 – 270). Numerical reference for amino acid substitutions is preferably based on the alignments of the A and B subunits of ADP-ribosylating toxins set forth in Domenighini *et al.*, Mol. Microbiol (1995) 15(6):1165 – 1167, specifically incorporated herein by reference in its entirety.

By way of further example, at least one of the entertoxin subunit coding regions may be genetically modified to detoxify the subunit peptide encoded thereby, for example wherein the truncated A subunit coding region has been genetically modified to disrupt or inactivate ADP-ribosyl transferase activity in the subunit peptide expression product (see, for example, WO 03/004055).

Thus, these results demonstrate that this genetic adjuvant is particularly desirable where an even more enhanced CMI response is desired. Other desirable genetic adjuvants include but are not limited to nucleotide sequences encoding IL-10, IL-12, IL-13, the interferons (IFNs) (for example, IFN-alpha, IFN-ss, and IFN-gamma), and preferred combinations thereof. Still other such biologically active factors that enhance the CMI response may be readily selected by one of skill in the art, and a suitable plasmid vector containing same constructed by known techniques.

Preferred further adjuvants include, but are not limited to, one or more of the following set forth below:

Mineral Containing Compositions

Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminium salts and calcium salts. The invention includes mineral salts such as hydroxides (*e.g.* oxyhydroxides), phosphates (*e.g.* hydroxyphoshpates, orthophosphates), sulphates, *etc.* {*e.g.* see chapters 8 & 9 of ref. Bush and Everett (2001) Int J Syst Evol Microbiol 51: 203-220), or mixtures of different mineral compounds, with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous, *etc.*), and with adsorption being preferred. The mineral containing compositions may also be formulated as a particle of metal salt. See WO 00/23105.

Aluminum salts may be included in immunogenic compositions and/or vaccines of the invention such that the dose of Al^{3+} is between 0.2 and 1.0 mg per dose.

Preferably the adjuvant is alum, preferably an aluminium salt such as aluminium hydroxide (AlOH) or aluminium phospate or aluminium sulfate. Still more preferably the adjuvant is aluminium hydroxide (AlOH).

Preferably a mineral salt, such as an aluminium salt, is combined with and another adjuvant, such as an oligonucleotide containing a CpG motif or an ADP ribosylating toxin. Still more preferably, the mineral salt is combined with an oligonucleotide containing a CpG motif.

Oil-Emulsions

Oil-emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). See WO90/14837. See also, Frey et al., "Comparison of the safety, tolerability, and immunogenicity of a MF59-adjuvanted influenza vaccine and a non-adjuvanted influenza vaccine in non-elderly adults", Vaccine (2003) 21:4234-4237. MF59 is used as the adjuvant in the FLUAD™ influenza virus trivalent subunit vaccine.

Particularly preferred adjuvants for use in the compositions are submicron oil-inwater emulsions. Preferred submicron oil-in-water emulsions for use herein are squalene/water emulsions optionally containing varying amounts of MTP-PE, such as a submicron oil-in-water emulsion containing 4-5% w/v squalene, 0.25-1.0% w/v Tween 80 ™ (polyoxyelthylenesorbitan monooleate), and/or 0.25-1.0% Span 85™ (sorbitan trioleate), and, optionally, N-acetylmuramyl-L-alanyl-D-isogluatminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-huydroxyphosphophoryloxy)-ethylamine (MTP-PE), for example, the submicron oil-in-water emulsion known as "MF59" (International Publication No. WO90/14837; US Patent Nos. 6,299,884 and 6,451,325, incorporated herein by reference in their entireties; and Ott et al., "MF59 -- Design and Evaluation of a Safe and Potent Adjuvant for Human Vaccines" in *Vaccine Design: The Subunit and Adjuvant Approach* (Powell, M.F. and Newman, M.J. eds.) Plenum Press, New York, 1995, pp. 277-296). MF59 contains 4-5% w/v Squalene (e.g. 4.3%), 0.25-0.5% w/v Tween 80™, and 0.5% w/v Span 85™ and optionally contains various amounts of MTP-PE, formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA). For example, MTP-PE may be present in an amount of about 0-500 μg/dose, more preferably 0-250 μg/dose and most preferably, 0-100 μg/dose. As used herein, the term "MF59-0" refers to the above submicron oil-in-water emulsion lacking MTP-PE, while the term MF59-MTP denotes a formulation that contains MTP-PE. For instance, "MF59-100" contains 100 μg MTP-PE per dose, and so on. MF69, another submicron oil-in-water emulsion for use herein, contains 4.3% w/v squalene, 0.25% w/v Tween 80™, and 0.75% w/v Span 85™ and optionally MTP-PE. Yet another submicron oil-in-water emulsion is MF75, also known as SAF, containing 10% squalene, 0.4% Tween 80™, 5% pluronic-blocked polymer L121, and thr-MDP, also microfluidized into a submicron emulsion. MF75-MTP denotes an MF75 formulation that includes MTP, such as from 100-400 μg MTP-PE per dose.

Submicron oil-in-water emulsions, methods of making the same and immunostimulating agents, such as muramyl peptides, for use in the compositions, are described in detail in International Publication No. WO90/14837 and US Patent Nos. 6,299,884 and 6,45 1,325, incorporated herein by reference in their entireties.

Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used as adjuvants in the invention.

Saponin Formulations

Saponin formulations, may also be used as adjuvants in the invention. Saponins are a heterologous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponin from the bark of the *Quillaia saponaria* Molina tree have been widely studied as adjuvants. Saponin can also be commercially obtained from *Smilax ornata* (sarsaprilla), *Gypsophilla paniculata* (brides veil), and *Saponaria officianalis* (soap root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs. Saponin compositions have been purified using High Performance Thin Layer Chromatography (HP-LC) and Reversed Phase High Performance Liquid Chromatography (RP-HPLC). Specific purified fractions using these techniques have been identified, including QS7, QS17, QS18, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. A method of production of QS21 is disclosed in U.S. Patent No. 5,057,540. Saponin formulations may also comprise a sterol, such as cholesterol (see WO 96/33739).

Combinations of saponins and cholesterols can be used to form unique particles called Immunostimulating Complexs (ISCOMs). ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. Preferably, the ISCOM includes one or more of Quil A, QHA and QHC. ISCOMs are further described in EP 0 109 942, WO 96/11711 and WO 96/33739. Optionally, the ISCOMS may be devoid of additional detergent. See WO 00/07621.

A review of the development of saponin based adjuvants can be found in Barr *et al* (1998) Advanced Drug Delivery Reviews 32: 247-271 and Sjolander *et al* (1998) Advanced Drug Delivery Reviews (1998) 32: 321-338.

Virosomes and Virus Like Particles (VLPs)

Virosomes and Virus Like Particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Qß-phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in WO 03/024480, WO 03/024481; Niikura *et al* Virology (2002) 293:273 – 280; Lenz *et al* Journal of Immunology (2001) 5246 – 5355; Pinto, *et al* Journal of Infectious Diseases (2003) 188:327 – 338; and Gerber *et al* Journal of Virology (2001) 75(10):4752 – 47601; Virosomes are discussed further in, for example, Gluck *et al* Vaccine (2002) 20:B10 –B16.

Bacterial or Microbial Derivatives

Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as:

Non-toxic derivatives of enterobacterial lipopolysaccharide (LPS)

Such derivatives include Monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL). 3dMPL is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in EP 0 689 454. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22 micron membrane (see EP 0 689 454). Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives *e.g.* RC-529. See Johnson *et al.* (1999) *Bioorg Med Chem Lett* 9:2273-2278.

Lipid A Derivatives
Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as OM-174. OM-174 is described for example in Meraldi *et al.* Vaccine (2003) 21:2485 – 2491; Pajak, *et al* Vaccine (2003) 21:836 – 842.

Immunostimulatory oligonucleotides
Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a sequence containing an unmethylated cytosine followed by guanosine and linked by a phosphate bond). Bacterial double stranded RNA or oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

The CpG's can include nucleotide modifications/analogs such as phosphorothioate modifications and can be double-stranded or single-stranded. Optionally, the guanosine may be replaced with an analog such as 2'-deoxy-7-deazaguanosine. See Kandimalla, *et al* Nucleic Acids Research (2003) 31(9): 2393 – 2400; WO 02/26757 and WO 99/62923 for examples of possible analog substitutions. The adjuvant effect of CpG oligonucleotides is further discussed in Krieg Nature Medicine (2003) 9(7): 831 – 835; McCluskie, *et al* FEMS Immunology and Medical Microbiology (2002) 32:179 – 185; WO 98/40100, U.S. Patent No. 6,207,646, U.S. Patent No. 6,239,116, and U.S. Patent No. 6,429,199.

The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT. See Kalman *et al* (1999) (Nature Genetics 21: 385-389). The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it may be more specific for inducing a B cell response, such a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in Blackwell, *et al* J. Immunol. (2003) 170(8):4061 – 4068; Krieg BBRC (2003) 306:948 – 953; and WO 01/95935. Preferably, the CpG is a CpG-A ODN.

Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form "immunomers". See, for example, Kandimalla, *et al* (2003) 31(part 3):664 – 658; Bhagat *et al* BBRC (2003) 300:853 – 861 and WO 03/035836.

Preferably the adjuvant is CpG. Even more preferably, the adjuvant is Alum and an oligonucleotide containg a CpG motif or AlOH and an oligonucleotide containing a CpG motif.

Human Immunomodulators
Human immunomodulators suitable for use as adjuvants in the invention include cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (*e.g.* interferon-γ), macrophage colony stimulating factor, and tumor necrosis factor.

ADP-ribosylating toxins and detoxified derivatives thereof.
Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. Preferably, the protein is derived from *E. coli* (i.e., E. coli heat labile enterotoxin "LT), cholera ("CT"), or pertussis ("PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in WO95/17211

and as parenteral adjuvants in WO98/42375. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63, LT-R72, and LTR192G. The use of ADP-ribosylating toxins and detoxified derivaties thereof, particularly LT-K63 and LT-R72, as adjuvants can be found in the following references, each of which is specifically incorporated by reference herein in their entirety: Beignon, et al., "The LTR72 Mutant of Heat-Labile Enterotoxin of Escherichia coli Enahnces the Ability of Peptide Antigens to Elicit CD4+ T Cells and Secrete Gamma Interferon after Coapplication onto Bare Skin", Infection and Immunity (2002) 70(6):3012-3019; Pizza, et al., "Mucosal vaccines: non toxic derivatives of LT and CT as mucosal adjuvants", Vaccine (2001) 19:2534-2541; Pizza, et al., "LTK63 and LTR72, two mucosal adjuvants ready for clinical trials" Int. J. Med. Microbiol (2000) 290(4-5):455-461; Scharton-Kersten et al., "Transcutaneous Immunization with Bacterial ADP-Ribosylating Exotoxins, Subunits and Unrelated Adjuvants", Infection and Immunity (2000) 68(9):5306-5313; Ryan et al., "Mutants of Escherichia coli Heat-Labile Toxin Act as Effective Mucosal Adjuvants for Nasal Delivery of an Acellular Pertussis Vaccine: Differential Effects of the Nontoxic AB Complex and Enzyme Activity on Th1 and Th2 Cells" Infection and Immunity (1999) 67(12):6270-6280; Partidos et al., "Heat-labile enterotoxin of Escherichia coli and its site-directed mutant LTK63 enhance the proliferative and cytotoxic T-cell responses to intranasally co-immunized synthetic peptides", Immunol. Lett. (1999) 67(3):209-216; Peppoloni et al., "Mutants of the Escherichia coli heat-labile enterotoxin as safe and strong adjuvants for intranasal delivery of vaccines", Vaccines (2003) 2(2):285-293; and Pine et al., (2002) "Intranasal immunization with influenza vaccine and a detoxified mutant of heat labile enterotoxin from Escherichia coli (LTK63)" J. Control Release (2002) 85(1-3):263-270. Numerical reference for amino acid substitutions is preferably based on the alignments of the A and B subunits of ADP-ribosylating toxins set forth in Domenighini et al., Mol. Microbiol (1995) 15(6):1165-1167, specifically incorporated herein by reference in its entirety.

Preferably the adjuvant is an ADP-ribosylating toxin and an oligonucleotide containing a CpG motif (see for example, WO 01/34185)

Preferably the adjuvant is a detoxified ADP-ribosylating toxin and an oligonucleotide containing a CpG motif.

Preferably the detoxified ADP-ribosylating toxin is LTK63 or LTK72.

Preferably the adjuvant is LTK63. Preferably the adjuvant is LTK72.

Preferably the adjuvant is LTK63 and an oligonucleotide containing a CpG motif.

Preferably the adjuvant is LTK72 and an oligonucleotide containing a CpG motif.

Bioadhesives and Mucoadhesives

Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres (Singh *et al.* (2001) *J. Cont. Rele.* 70:267-276) or mucoadhesives such as cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention. See for example, WO99/27960.

Microparticles

Microparticles may also be used as adjuvants in the invention. Microparticles (*i.e.* a particle of ~100nm to ~150μm in diameter, more preferably ~200nm to ~30μm in diameter, and most preferably ~500nm to ~10μm in diameter) formed from materials that are biodegradable and non-toxic (*e.g.* a poly(α-hydroxy acid), a

polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, *etc.*), with poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (*e.g.* with SDS) or a positively-charged surface (*e.g.* with a cationic detergent, such as CTAB).

Liposomes
Examples of liposome formulations suitable for use as adjuvants are described in U.S. Patent No. 6,090,406, U.S. Patent No. 5,916,588, and EP 0 626 169.

Polyoxyethylene ether and Polyoxyethylene Ester Formulations
Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters (WO99/52549). Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol (WO01/21207) as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol (WO01/21152). Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether (laureth 9), polyoxyethylene-9-steoryl ether, polyoxytheylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

Polyphosphazene (PCPP)
PCPP formulations are described, for example, in Andrianov *et al* Biomaterials (1998) 19(1 – 3):109 – 115; Payne *et al* Adv. Drug. Delivery Review (1998) 31(3):185 – 196.

Muramyl peptides
Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE).

Imidazoquinolone Compounds
Examples of imidazoquinolone compounds suitable for use adjuvants in the invention include Imiquamod and its homologues, described further in Stanley, "Imiquimod and the imidazoquinolones: mechanism of action and therapeutic potential" Clin Exp Dermatol (2002) 27(7):571 – 577; and Jones, "Resiquimod 3M", Curr Opin Investig Drugs (2003) 4(2):214 – 218. The invention may also comprise combinations of aspects of one or more of the adjuvants identified above. For example, the following adjuvant compositions may be used in the invention:

(1) a saponin and an oil-in-water emulsion (WO99/11241);

(2) a saponin (e.g.., QS21) + a non-toxic LPS derivative (e.g., 3dMPL) (see WO 94/00153);

(3) a saponin (e.g.., QS21) + a non-toxic LPS derivative (e.g., 3dMPL) + a cholesterol;

(4) a saponin (*e.g.* QS21) + 3dMPL + IL-12 (optionally + a sterol (WO98/57659);

combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (European patent applications 0835318, 0735898 and 0761231).

(5) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion.

(6) Ribi™ adjuvant system (RAS), (Ribi Immunochem) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); and

(7) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dPML).

(7) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (See European patent applications 0835318, 0735898 and 0761231);

(8) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dPML); and

(9) one or more mineral salts (such as an aluminum salt) + an immunostimulatory oligonucleotide (such as a nucleotide sequence including a CpG motif).

Aluminium salts and MF59 are preferred adjuvants for parenteral immunisation. Mutant bacterial toxins are preferred mucosal adjuvants. Bacterial toxins and bioadhesives are preferred adjuvants for use with mucosally-delivered vaccines, such as nasal vaccines.

The composition may include an antibiotic.

Preferably the compositions of the present invention are administered with alum and/or CpG sequences.

Nucleic Acid

The antigens or epitopes of the present invention may be administered as nucleotide sequences encoding the antigens or epitopes. As used herein, the term nucleotide sequence refers to one of more nucleotide sequences which encode one or more epitopes which are used in the compositions or combinations of the present invention. The term "nucleotide sequence (NOI)" is synonymous with the term "polynucleotide" or "nucleic acid". The NOI may be DNA or RNA of genomic or synthetic or of recombinant origin. The NOI may be double-stranded or single-stranded whether representing the sense or antisense strand or combinations thereof. For some applications, preferably, the NOI is DNA. For some applications, preferably, the NOI is prepared by use of recombinant DNA techniques (e.g. recombinant DNA). For some applications, preferably, the NOI is cDNA. For some applications, preferably, the NOI may be the same as the naturally occurring form.

The term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones (*e.g.* phosphorothioates, *etc.*), and also peptide nucleic acids (PNA), *etc.* The invention includes nucleic acid comprising sequences complementary to those described above (*e.g.* for antisense or probing purposes).

Nucleic acid according to the invention can be prepared in many ways (*e.g.* by chemical synthesis, from genomic or cDNA libraries, from the organism itself, *etc.*) and can take various forms (*e.g.* single stranded, double stranded, vectors, probes, *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other *Chlamydia*l or host cell nucleic acids).

The invention provides a process for producing nucleic acid of the invention, comprising the step of amplifying nucleic acid using a primer-based amplification method (*e.g.* PCR).

The invention provides a process for producing nucleic acid of the invention, comprising the step of synthesising at least part of the nucleic acid by chemical means.

VECTOR

In one embodiment of the present invention, an antigen or antigenic combination or NOI encoding same is administered directly to a host subject. In another embodiment of the present invention, a vector comprising an NOI is administered to a host subject. Preferably the NOI is prepared and/or administered using a genetic vector. As it is well known in the art, a vector is a tool that allows or faciliates the transfer of an entity from one environment to another. In accordance with the present invention, and by way of example, some vectors used in recombinant DNA techniques allow entities, such as a segment of DNA (such as a heterologous DNA segment, such as a heterologous cDNA segment), to be transferred into a host and/or a target cell for the purpose of replicating the vectors comprising the NOI of the present invention and/or expressing the antigens or epitopes of the present invention encoded by the NOI. Examples of vectors used in recombinant DNA techniques include but are not limited to plasmids, chromosomes, artificial chromosomes or viruses. The term "vector" includes expression vectors and/or transformation vectors. The term "expression vector" means a construct capable of *in vivo or in vitro/ex vivo* expression. The term "transformation vector" means a construct capable of being transferred from one species to another.

NAKED DNA

The vectors comprising the NOI of the present invention may be administered directly as "a naked nucleic acid construct", preferably further comprising flanking sequences homologous to the host cell genome. As used herein, the term "naked DNA" refers to a plasmid comprising the NOI of the present invention together with a short promoter region to control its production. It is called "naked" DNA because the plasmids are not carried in any delivery vehicle. When such a DNA plasmid enters a host cell, such as a eukaryotic cell, the proteins it encodes are transcribed and translated within the cell.

VIRAL VECTORS

Alternatively, the vectors comprising the NOI of the present invention may be introduced into suitable host cells using a variety of viral techniques which are known in the art, such as for example infection with recombinant viral vectors such as retroviruses, herpes simplex viruses and adenoviruses. The vector may be a recombinant viral vectors. Suitable recombinant viral vectors include but are not limited to adenovirus vectors, adeno-associated viral (AAV) vectors, herpes-virus vectors, a retroviral vector, lentiviral vectors, baculoviral vectors, pox viral vectors or parvovirus vectors (see Kestler *et al* 1999 Human Gene Ther 10(10):1619-32). In the case of viral vectors, administration of the NOI is mediated by viral infection of a target cell.

TARGETED VECTOR

The term "targeted vector" refers to a vector whose ability to infect or transfect or transduce a cell or to be expressed in a host and/or target cell is restricted to certain cell types within the host subject, usually cells having a common or similar phenotype.

EXPRESSION VECTOR

Preferably, the NOI of the present invention which is inserted into a vector is operably linked to a control sequence that is capable of providing for the expression of the antigens or epitopes by the host cell, i.e. the vector is an expression vector. The agent produced by a host cell may be secreted or may be contained intracellularly depending on the NOI and/or the vector used. As will be understood by those of skill in the art, expression vectors containing the NOI can be designed with signal sequences which direct secretion of the EOI through a particular prokaryotic or eukaryotic cell membrane.

FUSION PROTEINS

The *Chlamydia pneumoniae* antigens used in the invention may be present in the composition as individual separate polypeptides, but it is preferred that at least two (*i.e.* 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20) of the antigens are expressed as a single polypeptide chain (a 'hybrid' polypeptide). Hybrid polypeptides offer two principal advantages: first, a polypeptide that may be unstable or poorly expressed on its own can be assisted by adding a suitable hybrid partner that overcomes the problem; second, commercial manufacture is simplified as only one expression and purification need be employed in order to produce two polypeptides which are both antigenically useful.

The hybrid polypeptide may comprise two or more polypeptide sequences from the first antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, wherein said first and second amino acid sequences are selected from a *Chlamydia* bactgerium, preferably a *Chlamydia pneumoniae* antigen or a fragment thereof of the first antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise different epitopes.

The hybrid polypeptide may comprise two or more polypeptide sequences from the second antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, wherein said first and second amino acid sequences are selected from a *Chlamydia pneumoniae* antigen or a fragment thereof of the second antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise difference epitopes.

The hybrid polypeptide may comprise one or more polypeptide sequences from the first antigen group and one or more polypeptide sequences from the second antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, said first amino acid sequence selected from a *Chlamydia pneumoniae* antigen or a fragment thereof from the first antigen group and said second amino acid sequence selected from a *Chlamydia* bactgerium, preferably a *Chlamydia pneumoniae* antigen or a fragment thereof from the second antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise difference epitopes.

The hybrid polypeptide may comprise one or more polypeptide sequences from the first antigen group and one or more polypeptide sequences from the third antigen group or the fourth antigen group or the fifth antigen group or the sixth antigen group or the seventh antigen group or the eight antigen group or the ninth antigen group or the tenth antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, said first amino acid sequence selected from a *Chlamydia pneumoniae* antigen or a fragment thereof from the first antigen group and said second amino acid sequence selected from a *Chlamydia pneumoniae* antigen or a fragment thereof from the third antigen group or the fourth antigen group or the fifth antigen group or the sixth antigen group or the seventh antigen group or the eight antigen group or the ninth antigen group or the tenth antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise difference epitopes.

The hybrid polypeptide may comprise one or more polypeptide sequences from the second antigen group and one or more polypeptide sequences from the third antigen group or the fourth antigen group or the fifth antigen group or the sixth antigen group or the seventh antigen group or the eight antigen group or the ninth antigen group or the tenth antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, said first amino acid sequence selected from a *Chlamydia pneumoniae* antigen or a fragment thereof from the second antigen group and said second amino acid sequence selected from a *Chlamydia pneumoniae* antigen or a fragment thereof from the third antigen group or the fourth antigen group or the fifth antigen group or the sixth antigen group or the seventh antigen group or the eight antigen group or the ninth antigen group or the tenth antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise difference epitopes.

Hybrids consisting of amino acid sequences from two, three, four, five, six, seven, eight, nine, or ten *Chlamydia pneumoniae* antigens are preferred. In particular, hybrids consisting of amino acid sequences from two, three, four, or five *Chlamydia pneumoniae* antigens are preferred. Different hybrid polypeptides may be mixed together in a single formulation. Within such combinations, a *Chlamydia pneumoniae* antigen may be present in more than one hybrid polypeptide and/or as a non-hybrid polypeptide. It is preferred, however, that an antigen is present either as a hybrid or as a non-hybrid, but not as both.

Two-antigen hybrids for use in the invention may comprise any one of the combinations disclosed above.

Hybrid polypeptides can be represented by the formula NH_2-A-{-X-L-}$_n$-B-COOH, wherein: X is an amino acid sequence of a *Chlamydia pneumoniae* antigen or a fragment thereof from the first antigen group, the second antigen group or the third antigen group or the fourth antigen group or the fifth antigen group or the sixth antigen group or the seventh antigen group or the eight antigen group or the ninth antigen group or the tenth antigen group.; L is an optional linker amino acid sequence; A is an optional N-terminal amino acid sequence; B is an optional C-terminal amino acid sequence; and *n* is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15.

If a -X- moiety has a leader peptide sequence in its wild-type form, this may be included or omitted in the hybrid protein. In some embodiments, the leader peptides will be deleted except for that of the -X- moiety located at the N-terminus of the hybrid protein *i.e.* the leader peptide of X_1 will be retained, but the leader peptides of X_2 ... X_n will be omitted. This is equivalent to deleting all leader peptides and using the leader peptide of X_1 as moiety -A-.

For each *n* instances of {-X-L-}, linker amino acid sequence -L- may be present or absent. For instance, when *n*=2 the hybrid may be NH_2-X_1-L_1-X_2-L_2-COOH, NH_2-X_1-X_2-COOH, NH_2-X_1-L_1-X_2-COOH, NH_2-X_1-X_2-L_2-COOH, *etc.* Linker amino acid sequence(s) -L- will typically be short (*e.g.* 20 or fewer amino acids *i.e.* 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples comprise short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* comprising Gly_n where *n* = 2, 3, 4, 5, 6, 7, 8, 9, 10 or more), and histidine tags (*i.e.* His_n where *n* = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. A useful linker is GSGGGG (SEQ ID No 77), with the Gly-Ser dipeptide being formed from a *Bam*HI restriction site, thus aiding cloning and manipulation, and the $(Gly)_4$ tetrapeptide being a typical poly-glycine linker.

-A- is an optional N-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct protein trafficking, or short peptide sequences which facilitate cloning or purification (*e.g.* histidine tags *i.e.* His_n where *n* = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable N-terminal amino acid sequences will be apparent to those skilled in the art. If X_1 lacks its own N-terminus methionine, -A- is preferably an oligopeptide (*e.g.* with 1, 2, 3, 4, 5, 6, 7 or 8 amino acids) which provides a N-terminus methionine.

-B- is an optional C-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include sequences to direct protein trafficking, short peptide sequences which facilitate cloning or purification (*e.g.* comprising histidine tags *i.e.* His_n where *n* = 3, 4, 5, 6, 7, 8, 9, 10 or more), or sequences which enhance protein stability. Other suitable C-terminal amino acid sequences will be apparent to those skilled in the art. Most preferably, *n* is 2 or 3.

The invention also provides nucleic acid encoding hybrid polypeptides of the invention. Furthermore, the invention provides nucleic acid which can hybridise to this nucleic acid, preferably under "high stringency" conditions (*e.g.* 65°C in a 0.1xSSC, 0.5% SDS solution).

The NOI of the present invention may be expressed as a fusion protein comprising an adjuvant and/or a biological response modifier and/or immunomodulator fused to the antigens or epitopes of the present invention to further enhance and/or augment the CMI response obtained. The biological response modifier may act as an adjuvant in the sense of providing a generalised stimulation of the CMI response. The antigens or epitopes may be attached to either the amino or carboxy terminus of the biological response modifier.

METHODS OF MAKING

Polypeptides of the invention can be prepared by various means (*e.g.* recombinant expression, purification from cell culture, chemical synthesis, *etc.*) and in various forms (*e.g.* native, fusions, non-glycosylated, lipidated, *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other *Chlamydia*l or host cell proteins).

The invention also provides a process for producing a polypeptide of the invention, comprising the step of culturing a host cell transformed with nucleic acid of the invention under conditions which induce polypeptide expression. The invention provides a process for producing a polypeptide of the invention, comprising the step of synthesising at least part of the polypeptide by chemical means. The invention further provides a process for producing a composition according to the invention comprising the step of bringing one or more of SEQ IDs 1-86 into combination with one or more other of SEQ IDs 1-86

Strains

Preferred polypeptides of the invention comprise an amino acid sequence found in *C.pneumoniae* serovars, or in one or more of an epidemiologically prevalent serotype. Where hybrid polypeptides are used, the individual antigens within the hybrid (*i.e.* individual -X- moieties) may be from one or more strains. Where n=2, for instance, X_2 may be from the same strain as X_1 or from a different strain. Where n=3, the strains might be (i) $X_1=X_2=X_3$ (ii) $X_1=X_2\neq X_3$ (iii) $X_1\neq X_2=X_3$ (iv) $X_1\neq X_2\neq X_3$ or (v) $X_1=X_3\neq X_2$, *etc.*

Heterologous host

Whilst expression of the polypeptides of the invention may take place in *Chlamydia*, the invention preferably utilises a heterologous host. The heterologous host may be prokaryotic (*e.g.* a bacterium) or eukaryotic. It is preferably *E.coli*, but other suitable hosts include *Bacillus subtilis, Vibrio cholerae, Salmonella typhi, Salmonella typhimurium, Neisseria lactamica, Neisseria cinerea, Mycobacteria* (*e.g. M.tuberculosis*), yeasts, *etc.*

Details as to how the molecules which make up the SEQ IDs 1-86 can be produced and used can be found from the relevant international applications such as WO 00/37494, WO 02/02606 and WO 03/049762 and WO 03/068811 and these details need not be repeated here. Where the composition includes a protein that exists in different nascent and mature forms, the mature form of the protein is preferably used. For example, the mature form of the *Chlamydia pneumoniae* protein lacking the signal peptide may be used

ADMINISTRATION

Compositions of the invention will generally be administered directly to a patient. Direct delivery may be accomplished by parenteral injection (*e.g.* subcutaneously, intraperitoneally, intravenously, intramuscularly, or to the interstitial space of a tissue), or by rectal, oral (*e.g.* tablet, spray), vaginal, topical, transdermal {*e.g.* see WO99/27961) or transcutaneous {*e.g.* WO02/074244 and WO02/064162 intranasal {*e.g.* see WO03/028760) ocular, aural, pulmonary or other mucosal administration. The invention may be used to elicit systemic and/or mucosal immunity.

The compositions of the present invention may be administered, either alone or as part of a composition, via a variety of different routes. Certain routes may be favoured for certain compositions, as resulting in the generation of a more effective immune response, prefereably a CMI response, or as being less likely to induce side effects, or as being easier for administration.

By way of example, the compositions of the present invention may be administered via a systemic route or a mucosal route or a transdermal route or it may be administrered directly into a specific tissue. As used herein, the term "systemic administration" includes but is not limited to any parenteral routes of administration. In particular, parenteral administration includes but is not limited to subcutaneous, intraperitoneal, intravenous, intraarterial, intramuscular, or intrasternal injection, intravenous, intraarterial, or kidney dialytic infusion techniques. Preferably, the systemic, parenteral administration is intramuscular injection.

In one preferred embodiment of the method, the compositions of the present invention are administered via a transdermal route. While it is believed that any accepted mode and route of immunization can be employed and nevertheless achieve some advantages in accordance herewith, the examples below demonstrate particular advantages with transdermal NOI administration. In this regard, and without being bound by theory, it is believed that transdermal administration of a composition may be preferred because it more efficiently activates the cell mediated immune (CMI) arm of the immune system.

The term "transdermal" delivery intends intradermal (e.g., into the dermis or epidermis), transdermal (e.g.,"percutaneous") and transmucosal administration, i.e., delivery by passage of an agent into or through skin or mucosal tissue. *See, e.g., Transdermal Drug Delivery: Developmental Issues and Research Initiatives*, Hadgraft and Guy (eds.), Marcel Dekker, Inc., (1989); *Controlled Drug Delivery: Fundamentals and Applications*, Robinson and Lee (eds.), Marcel Dekker Inc.,(1987); and *Transdermal Delivery of Drugs*, Vols. 1-3, Kydonieus and Berner (eds.), CRC Press, (1987). Thus, the term encompasses delivery of an agent using a particle delivery device (e.g., a needleless syringe) such as those described in U.S. Patent No. 5,630,796, as well as delivery using particle-mediated delivery devices such as those described in U.S. Patent No. 5,865,796.

As used herein, the term "mucosal administration" includes but is not limited to oral, intranasal, intravaginal, intrarectal, intratracheal, intestinal and ophthalmic administration.

Mucosal routes, particularly intranasal, intratracheal, and ophthalmic are preferred for protection against natural exposure to environmental pathogens such as RSV, flu virus and cold viruses or to allergens such as grass and ragweed pollens and house dust mites. The enhancement of the immune response, preferably the CMI response will enhance the protective effect against a subsequently encountered target antigen such as an allergen or microbial agent.

In another preferred embodiment of the present invention, the compositions of the present invention may be administered to cells which have been isolated from the host subject. In this preferred embodiment, preferably the composition is administered to professional antigen presenting cells (APCs), such as dendritic cells. APCs may be derived from a host subject and modified *ex vivo* to express an antigen of interest and

then transferred back into the host subject to induce an enhanced CMI response. Dendritic cells are believed to be the most potent APCs for stimulating enhanced CMI responses because the expressed epitopes of the antigen of interest must be acquired, processed and presented by professional APCs to T cells (both Th1 and Th2 helper cells as well as CD8+ T-cells) in order to induce an enhanced CMI response.

PARTICLE ADMINISTRATION

Particle-mediated methods for delivering the compositions of the present invention are known in the art. Thus, once prepared and suitably purified, the above-described antigens or NOI encoding same can be coated onto core carrier particles using a variety of techniques known in the art. Carrier particles are selected from materials which have a suitable density in the range of particle sizes typically used for intracellular delivery from a gene gun device. The optimum carrier particle size will, of course, depend on the diameter of the target cells.

By "core carrier"" is meant a carrier on which a guest antigen or guest nucleic acid (e.g., DNA, RNA) is coated in order to impart a defined particle size as well as a sufficiently high density to achieve the momentum required for cell membrane penetration, such that the guest molecule can be delivered using particle-mediated techniques (see, e.g., U.S. Patent No. 5,100,792). Core carriers typically include materials such as tungsten, gold, platinum, ferrite, polystyrene and latex. See e.g., *Particle Bombardment Technology for Gene Transfer*, (1994) Yang, N. ed., Oxford University Press, New York, NY pages 10-11. Tungsten and gold particles are preferred. Tungsten particles are readily available in average sizes of 0.5 to 2.0 microns in diameter. Gold particles or microcrystalline gold (e. g., gold powder A1570, available from Engelhard Corp., East Newark, NJ) will also find use with the present invention. Gold particles provide uniformity in size (available from Alpha Chemicals in particle sizes of 1-3 microns, or available from Degussa, South Plainfield, NJ in a range of particle sizes including 0.95 microns). Microcrystalline gold provides a diverse particle size distribution, typically in the range of 0.5-5 microns. However, the irregular surface area of microcrystalline gold provides for highly efficient coating with nucleic acids. A number of methods are known and have been described for coating or precipitating NOIs onto gold or tungsten particles. Most such methods generally combine a predetermined amount of gold or tungsten with plasmid DNA, CaCl2 and spermidine. The resulting solution is vortexed continually during the coating procedure to ensure uniformity of the reaction mixture. After precipitation of the NOI, the coated particles can be transferred to suitable membranes and allowed to dry prior to use, coated onto surfaces of a sample module or cassette, or loaded into a delivery cassette for use in particular gene gun instruments.

The particle compositions or coated particles are administered to the individual in a manner compatible with the dosage formulation, and in an amount that will be effective for the purposes of the invention. The amount of the composition to be delivered (e. g., about 0.1 mg to 1 mg, more preferably 1 to 50 ug of the antigen or allergen, depends on the individual to be tested. The exact amount necessary will vary depending on the age and general condition of the individual to be treated, and an appropriate effective amount can be readily determined by one of skill in the art upon reading the instant specification.

HOST MAMMALIAN SUBJECT

As used herein, the term "host mammalian subject" means any member of the subphylum cordata, including, without limitation, humans and other primates, including non-human primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs; birds, including domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like. The terms do not denote a particular age. Thus, both adult and newborn individuals are intended to be covered. The methods described herein are intended for use in any of the above vertebrate species, since the immune systems of all of these vertebrates operate similarly. If a mammal, the subject will preferably be a human, but may also be a domestic livestock, laboratory subject or pet animal.

The mammal is preferably a human. Where the vaccine is for prophylactic use, the human is preferably a child (*e.g.* a toddler or infant) or a teenager; where the vaccine is for therapeutic use, the human is preferably a teenager or an adult. A vaccine intended for children may also be administered to adults *e.g.* to assess safety, dosage, immunogenicity, *etc.*

PREVENT AND/OR TREAT

The invention also provides the use of the compositions of the invention in the manufacture of a medicament for raising an immune response in a mammal. The medicament is preferably a vaccine and to the preparation of a vaccine to prevent and/or treat an disorder associated with a *Chlamydia* bacterium. It is to be appreciated that all references herein to treatment include curative, palliative and prophylactic treatment.

The administration of antigenic combinations of the present invention or a composition comprising the NOI encoding the antigenic combinations may be for either "prophylactic" or "therapeutic" purpose. As used herein, the term "therapeutic" or "treatment" includes any of following: the prevention of infection or reinfection; the reduction or elimination of symptoms; and the reduction or complete elimination of a pathogen. Treatment may be effected prophylactically (prior to infection) or therapeutically (following infection).

Prophylaxis or therapy includes but is not limited to eliciting an effective immune response, preferably a CMI immune response and/or alleviating, reducing, curing or at least partially arresting symptoms and/or complications resulting from a T cell mediated immune disorder. When provided prophylactically, the composition of the present invention is typically provided in advance of any symptom. The prophylactic administration of the composition of the present invention is to prevent or ameliorate any subsequent infection or disease. When provided therapeutically, the composition of the present invention is typically provided at (or shortly after) the onset of a symptom of infection or disease. Thus the composition of the present invention may be provided either prior to the anticipated exposure to a disease causing agent or disease state or after the initiation of an infection or disease.

Whether prophylactic or therapeutic administration (either alone or as part of a composition) is the more appropriate will usually depend upon the nature of the disease. By way of example, immunotherapeutic composition of the present invention could be used in immunotherapy protocols to actively inducing immunity

by vaccination. This latter form of treatment is advantageous because the immunity is prolonged. On the other hand a vaccine composition will preferably, though not necessarily be used prophylactically to induce an effective CMI response against subsequently encountered antigens or portions thereof (such as epitopes) related to the target antigen.

These uses and methods are preferably for the prevention and/or treatment of a disease caused by a *Chlamydia* (*e.g.* trachoma, pelvic inflammatory disease, epididymitis, infant pneumonia, artherosclerosis, cardiovascular disease *etc.*). The compositions may also be effective against *C.pneumoniae*.

PROPHYLACTICALLY OR THERAPEUTICALLY OR IMMUNOLOGICALLY EFFECTIVE AMOUNT

The composition dose administrated to a host subject, in the context of the present invention, should be sufficient to effect a beneficial prophylactic or therapeutic immune response, preferably a CMI response in the subject over time.

The invention also provides a method for raising an immune response in a mammal comprising the step of administering an effective amount of a composition of the invention. The immune response is preferably protective and preferably involves antibodies and/or cell-mediated immunity. The method may raise a booster response.

As used herein, the term ""prophylactically or therapeutically effective dose" means a dose in an amount sufficient to elicit an enhanced immune response, preferably a CMI response to one or more antigens or epitopes and/or to alleviate, reduce, cure or at least partially arrest symptoms and/or complications from a T cell mediated immune disorder.

Immunogenic compositions used as vaccines comprise an immunologically effective amount of antigen(s), as well as any other components, as needed. By 'immunologically effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.* non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

The mammal is preferably a human. Where the vaccine is for prophylactic use, the human is preferably a child (e.g. a toddler or infant) or a teenager or an adult; where the vaccine is for therapeutic use, the human is preferably a teenager or an adult. A vaccine intended for children may also be administered to adults e.g. to assess safety, dosage, immunogenicity, etc. Preferably, the human is a teenager. More preferably, the human is a pre-adolescent teenager. Even more preferably, the human is a pre-adolescent female or male Preferably the pre-adolescent male or female is around 9-12 years of age.

One way of assessing the immunogenicity of the component proteins of the immunogenic compositions of the present invention is to express the proteins recombinantly and to screen patient sera or mucosal secretions by immunoblot or by protein or DNA microarray. A positive reaction between the protein and the patient serum indicates that the patient has previously mounted an immune response to the protein in question- that is, the protein is an immunogen. This method may also be used to identify immunodominant proteins.

One way of checking efficacy of therapeutic treatment involves monitoring *Chlamydia* infection after administration of the composition of the invention. One way of checking efficacy of prophylactic treatment involves monitoring immune responses against the *Chlamydia antigen,* such as the *Chlamydia pneumoniae* antigen in the compositions of the invention after administration of the composition. For example, checking efficacy of prophylactic treatment may involve monitoring immune responses both systemically (such as monitoring the level of IgG1 and IgG2a production) and mucosally (such as monitoring the level of IgA production) against the *Chlamydia pneumoniae* antigens in the compositions of the invention after administration of the composition. Typically, serum *Chlamydia* specific antibody responses are determined post-immunization but pre-challenge whereas mucosal *Chlamydia* specific antibody body responses are determined post-immunization and post-challenge.

These uses and methods are preferably for the prevention and/or treatment of a disease caused by *Chlamydia pneumoniae* (e.g. pneumonia, bronchitis, pharyngitis, sinusitis, erythema nodosum, asthma, atherosclerosis, stroke, myocardial infarctions, coronary artery disease, etc.).

The vaccine compositions of the present invention can be evaluated in *in vitro* and *in vivo* animal models prior to host, e.g., human, administration. For example, *in vitro* neutralization by Peterson *et al* (1988) is suitable for testing vaccine compositions directed toward *Chlamydia,* preferably *Chlamydia pneumoniae.*

One example of such an *in vitro* test is described as follows. Hyper-immune antisera is diluted in PBS containing 5% guinea pig serum, as a complement source. *Chlamydia pneumoniae* (10^4 IFU; inclusion forming units) are added to the antisera dilutions. The antigen-antibody mixtures are incubated at 37°C for 45 minutes and inoculated into duplicate confluent Hep-2 or HeLa cell monolayers contained in glass vials (e.g., 15 by 45 mm), which have been washed twice with PBS prior to inoculation. The monolayer cells are infected by centrifugation at 1000X g for 1 hour followed by stationary incubation at 37°C for 1 hour. Infected monolayers are incubated for 48 or 72 hours, fixed and stained with *Chlamydia* specific antibody, such as anti-MOMP. Inclusion-bearing cells are counted in ten fields at a magnification of 200X. Neutralization titer is assigned on the dilution that gives 50% inhibition as compared to control monolayers/IFU.

The efficacy of immunogenic compositions can also be determined in vivo by challenging animal models of Chlamydia pneumoniae infection, e.g., guinea pigs or mice, with the immunogenic compositions. The immunogenic compositions may or may not be derived from the same serovars as the challenge serovars. Preferably the immunogenic compositions are derivable from the same serovars as the challenge serovars. More preferably, The serovars of the present invention are obtainable from clinical isolates or from culture collections such as the American Tissue Culture Collection (ATCC).

In vivo efficacy models include but are not limited to: (i) A murine infection model using human Chlamydia pneumoniae serotypes; (ii) a murine disease model which is a murine model using a mouse-adapted Chlamydia pneumoniae strain, such as the Chlamydia pneumoniae mouse pneumonitis (MoPn) strain also known as Chlamydia muridarum; and (iii) a primate model using human Chlamydia pneumoniae isolates. The MoPn strain is a mouse pathogen while human Chlamydia pneumoniae serotypes are human pathogens (see for example, Brunham et al (2000) J Infect Dis 181 (Suppl 3) S538-S543; Murdin et al (2000) J Infect Dis 181 (Suppl 3) S544-S551 and Read et al (2000) NAR 28(6); 1397-1406). As the Examples demonstrate, human Chlamydia pneumoniae serotypes can be used in mouse models although they normally require high inocula or pretreatment with progesterone. Progesterone is generally used because it seems to render the epithelium more susceptible to chlamydial infection (see Pal et al 2003 Vaccine 21: 1455-1465). One the other hand, MoPn, which was originally isolated from mouse tissues, is thought to be a natural murine pathogen and thus offers an evolutionarily adapted pathogen for analysis of host-pathogen interactions. Although the MoPn serovar is thought to have a high degree of DNA homology to the human Chlamydia serovars, it may also have some unique properties (see for example, Pal et al (2002) Infection and Immunity 70(9); 4812-4817.

By way of example, in vivo vaccine compositions challenge studies can be performed in the murine model of Chlamydia pneumoniae (Morrison et al 1995). A description of one example of this type of approach is as follows. Female mice 7 to 12 weeks of age receive 2.5 mg of depoprovera subcutaneously at 10 and 3 days before vaginal infection. Post-vaccination, mice are infected in the genital tract with 1,500 inclusion-forming units of Chlamydia pneumoniae contained in 5ml of sucrose-phosphate-glutamate buffer, pH 7.4. The course of infection is monitored by determining the percentage of inclusion-bearing cells by indirect immunofluorescence with Chlamydia pneumoniae specific antisera, or by a Giemsa-stained smear from a scraping from the genital tract of an infected mouse. The presence of antibody titers in the serum of a mouse is determined by an enzyme-linked immunosorbent assay. The immunogenic compositions of the present invention can be administered using a number of different immunization routes such as but not limited to intra-muscularly (i.m.), intra-peritoneal (i.p.), intra-nasal (i.n.), sub-cutaneous (s.c) or transcutaneous (t.c) routes. Generally, any route of administration can be used provided that the desired immune response at the required mucosal surface or surfaces is achieved. Likewise, the challenge serovars may be administered by a number of different routes. Typically, the challenge serovars are administered mucosally, such as but not limited to an intra-nasal (i.n) challenge.

Alternative in-vivo efficacy models include guinea pig models. For example, in vivo vaccine composition challenge studies in the guinea pig model of Chlamydia

pneumoniae infection can be performed. A description of one example of this type of approach follows. Female guinea pigs weighing 450 – 500 g are housed in an environmentally controlled room with a 12 hour light-dark cycle and immunized with vaccine compositions via a variety of immunization routes. Post-vaccination, guinea pigs are infected in the genital tract with the agent of guinea pig inclusion conjunctivitis (GPIC), which has been grown in HeLa or McCoy cells (Rank et al. (1988)). Each animal receives approximately $1.4x10^7$ inclusion forming units (IFU) contained in 0.05 ml of sucrose-phosphate-glutamate buffer, pH 7.4 (Schacter, 1980). The course of infection monitored by determining the percentage of inclusion-bearing cells by indirect immunofluorescence with GPIC specific antisera, or by Giemsa-stained smear from a scraping from the genital tract (Rank et al 1988). Antibody titers in the serum is determined by an enzyme-linked immunosorbent assay.

Compositions of the invention will generally be administered directly to a patient. Direct delivery may be accomplished by parenteral injection (e.g. subcutaneously, intraperitoneally, intravenously, intramuscularly, or to the interstitial space of a tissue), or mucosally, such as by rectal, oral (e.g. tablet, spray), vaginal, topical, transdermal (See e.g. WO99/27961) or transcutaneous (See e.g. WO02/074244 and WO02/064162), intranasal (See e.g. WO03/028760), ocular, aural, pulmonary or other mucosal administration.

DOSAGE

Prophylaxis or therapy can be accomplished by a single direct administration at a single time point or multiple time points. Administration can also be delivered to a single or to multiple sites. Some routes of administration, such as mucosal administration via ophthalmic drops may require a higher dose. Those skilled in the art can adjust the dosage and concentration to suit the particular route of delivery.

Dosage treatment can be a single dose schedule or a multiple dose schedule. multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule. in a multiple dose schedule the various doses may be given by the same or different routes *e.g.* a parenteral prime and mucosal boost, a mucosal prime and parenteral boost, *etc.*

HOMOLOGUES

SEQ IDs 1-86 in the compositions of the invention may be supplemented or substituted with molecules comprising sequences homologous (ie. sharing sequence identitv) to SEQ ID Nos 1-86.

Proteins (including protein antigens) as used in the invention (as encoded by the NOI) may have homology and/or sequence identity with naturally occurring forms. Similarly coding sequences capable of expressing such proteins will generally have homology and/or sequence identity with naturally occurring sequences. Techniques for determining nucleic acid and amino acid "sequence identity" also are known in the art. Typically, such techniques include determining the nucleotide sequence of the mRNA for a gene and/or determining the amino acid sequence encoded thereby, and comparing these sequences to a second nucleotide or amino acid sequence.

In general, "identity" refers to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences,

respectively. Two or more sequences (polynucleotide or amino acid) can be compared by determining their "percent identity." The percent identity of two sequences, whether nucleic acid or amino acid sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequences and multiplied by 100.

An approximate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2: 482-489 (1981). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff, Atlas of Protein Sequences and Structure, M. O. Dayhoff ed., 5 suppl. 3: 353-358, National Biomedical ResearchFoundation, Washington, D. C., USA, and normalized by Gribskov, Nucl. AcidsRes. 14 (6): 6745-6763 (1986). An exemplary implementation of this algorithm to determine percent identity of a sequence is provided by the Genetics Computer Group (Madison, WI) in the"BestFit"utility application. The default parameters for this method are described in the Wisconsin Sequence Analysis Package Program Manual, Version 8 (1995) (available from Genetics Computer Group, Madison, WI). A preferred method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S.

Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, CA). From this suite of packages the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six). From the data generated the "Match"value reflects"sequence identity." Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code = standard; filter = none; strand = both; cutoff= 60; expect = 10; Matrix = BLOSUM62 ; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank +EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs can be found at the following internet address: http://www. ncbi. nlm. gov/cgi-bin/BLAST.

Alternatively, homology can be determined by hybridization of polynucleotides under conditions which form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease (s), and size determination of the digested fragments. Two DNA, or two polypeptide sequences are "substantially homologous" to each other when the sequences exhibit at least about 80%-85%, preferably at least about 90%, and most preferably at least about 95%-98% sequence identity over a defined length of the molecules, as determined using the methods above.

As used herein, substantially homologous or homologous also refers to sequences showing complete identity to the specified DNA or polypeptide sequence. DNA sequences that are substantially homologous or homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. For example, stringent hybridization conditions can include 50% formamide, 5x Denhardt's Solution, 5x SSC, 0.1% SDS and 100 pg/ml

denatured salmon sperm DNA and the washing conditions can include 2x SSC, 0.1% SDS at 37 C followed by lx SSC, 0.1% SDS at 68 C. Defining appropriate hybridization conditions is within the skill of the art.

Preferably the degree of identity is preferably greater than 50% (eg. 65%. 80%. 90%. or more) and include mutants and allelic variants. Sequence identitv between the proteins is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford. Molecular). using an affine gap search with parameters gap open penalty=12 and gap extension penalty=1.

SEQ IDs 1-86 in the compositions of the invention may be supplemented or substituted with nucleic acid which can hybridise to the *Chlamydia* nucleic acid. preferably underv"high stringency"conditionsv(c. 65 C in an 0.1 x SSC, 0.5% SDS solution).

Hypothetical Protein

As used herein, the term "hypothetical protein" refers to a protein which lacks a known cellular location or a known cellular function. Typically, a hypothetical protein lacks significant homologies with known well characterised proteins.

COMPOSITIONS

The invention also provides the compositions of the invention for use as medicaments (eg. as immunogenic compositions or vaccines) or as diagnostic reagents for detecting a Chylamydia infectioin in a host subject. It also provides the use of the compositions in the manufacture of: (i) a medicament for treating or preventing infection due to *Chlamydia pneumoniae* bacteria: (ii) a diagnostic reagent for detecting the presence of *Chlamydia* Pneumonaie bacteria or of antibodies raised against *Chlamydia* Pneumonaie bacteria; and/or (iii) a reagent which can raise antibodies against *Chlamydia pneumonaie* bacteria.

The invention also provides a method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to the invention.

The present invention provides compositions that are useful for preventing and/or treating T cell mediated immune disorders. In one embodiment, the composition is a pharmaceutical composition. In another preferred embodiment, the composition is an immunotherapeutic composition. In an even more preferred embodiment, the composition is a vaccine composition.. The composition may also comprise a carrier such as a pharmaceutically or immunologically acceptable carrier. Pharmaceutically acceptable carriers or immunologically acceptable carriers are determined in part by the particular composition being administered as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions or vaccine compositions or immunotherapeutic compositions of the present invention.

Immunogenic compositions and medicaments

Compositions of the invention are preferably immunogenic compositions, and are more preferably vaccine compositions. The pH of the composition is preferably between 6 and 8, preferably about 7. The pH may be maintained by the use of a

buffer. The composition may be sterile and/or pyrogen-free. The composition may be isotonic with respect to humans.

Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection), but will typically be prophylactic. Accordingly, the invention includes a method for the therapeutic or prophylactic treatment of *Chlamydia pneumoniae* infection in an animal susceptible to *Chlamydia*l infection comprising administering to said animal a therapeutic or prophylactic amount of the immunogenic compositions of the invention. Preferably, the immunogenic composition comprises a combination of *Chlamydia pneumoniae* antigens, said combination selected from the group consisting of two, three, four, five or all six *Chlamydia pneumoniae* antigens of the first antigen group. Still more preferably, the combination consists of all six *Chlamydia pneumoniae* antigens of the first antigen group.

Alternatively, the immunogenic composition comprises a combination of *Chlamydia pneumoniae* antigens, said combination selected from the group consisting of two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve *Chlamydia pneumoniae* antigens selected from the first antigen group and the second antigen group. Preferably, the combination is selected from the group consisting of three, four, or five *Chlamydia pneumoniae* antigens selected from the second antigen group. Still more preferably, the combination consists of five *Chlamydia pneumoniae* antigens selected from the second antigen group.

Alternatively, the immunogenic composition comprises a combination of *Chlamydia pneumoniae* antigens, said combination consisting of two, three, four, or five *Chlamydia pneumoniae* antigens of the first antigen group and one, two, three, four, five or six *Chlamydia pneumoniae* antigens of the third antigen group. Preferably, the combination consists of three, four or five *Chlamydia pneumoniae* antigens of the first antigen group and one, two, three, four, five or six *Chlamydia pneumoniae* antigens of the third antigen group.

Alternatively, the immunigenic composition comprises a combination of *Chlamydia pneumoniae* antigens, said combination consisting of two, three, four, five, six, seven, eight, nine, ten, eleven or twelve *Chlamydia pneumoniae* antigens of the first antigen group and the second antigen group and one, two, three, four, five or six *Chlamydia pneumoniae* antigens of the third antigen group. Preferably, the combination is selected from the group consisting of three, four, or five *Chlamydia pneumoniae* antigens from the second antigen group and three, four or five *Chlamydia pneumoniae* from the third antigen group. Still more preferably, the combination consists of five *Chlamydia pneumoniae* antigens from the second antigen group and three, four or five *Chlamydia pneumoniae* antigens of the third antigen group.

In certain embodiments. the composition comprises molecules from different *Chlamydia* species. In some embodiments. the composition may comprise molecules from different serogroups and/or strains of the same *Chlamydia* species. Further embodiments comprise mixtures of one or more *Chlamydia* molecules from different strains.

Many proteins are relatively conserved between different species serogroups and

strains of *Chlamydia trachomatis* and *Chlamydia pneumoniae*. To ensure maximum cross-strain recognition and reactivity. regions of proteins that are conserved between different *Chlamydia* species, serogroups and strains can be used in the compositions of the present invention. The invention therefore provides proteins which comprise stretches of amino acid sequence that are shared across the majority of *Chlamydia* strains. Preferablv, therefore, the composition comprises a protein comprising a fragment of a *Chlamydia pneumoniae* protein (preferably a protein from SEQ ID Nos 1-86 or more preferably SEQ ID Nos 1-41 wherein said fragment consists of n consecutive conserved amino acids.

Further antigens

The compositions of the invention may further comprise antigen derived from one or more sexually transmitted diseases in addition to *Chlamydia trachomatis*. Preferably the antigen is derived from one or more of the following sexually transmitted diseases: *N.gonorrhoeae* {*e.g.* i, ii, iii, iv}; human papiloma virus; *Treponema pallidum;* herpes simplex virus (HSV-1 or HSV-2); HIV (HIV-1 or HIV-2); and *Haemophilus ducreyi.*

A preferred composition comprises: (1) at least *t* of the *Chlamydia pneumoniae* antigens from either the first antigen group or the second antigen group, where *t* is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13, preferably *t* is five; (2) one or more antigens from another sexually transmitted disease. Preferably, the sexually transmitted disease is selected from the group consisting of herpes simplex virus, preferably HSV-1 and/or HSV-2; human papillomavirus; *N.gonorrhoeae*; *Treponema pallidum*; and *Haemophilus ducreyi.* These compositions can thus provide protection against the following sexually-transmitted diseases: *Chlamydia*, genital herpes, genital warts, gonorrhoea, syphilis and chancroid (see Stephens et al (1998) Science 282: 754-759).

Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier protein in order to enhance immunogenicity (For example, Ramsay *et al.* (2001) *Lancet* 357(9251):195-196; Lindberg (1999) *Vaccine* 17 Suppl 2:S28-36; Buttery & Moxon (2000) *J R Coll Physicians Lond* 34:163-168; Ahmad & Chapnick (1999) *Infect Dis Clin North Am* 13:113-133; Goldblatt (1998) *J. Med. Microbiol.* 47:563-567; European patent 0 477 508; US Patent No. 5,306,492; International patent application WO98/42721; *Conjugate Vaccines* (eds. Cruse *et al.*) ISBN 3805549326, particularly vol. 10:48-114; and Hermanson (1996) *Bioconjugate Techniques* ISBN: 0123423368 or 012342335).

Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria or tetanus toxoids. The CRM_{197} diphtheria toxoid is particularly preferred (*Research Disclosure*, 453077 (Jan 2002). Other carrier polypeptides include the *N.meningitidis* outer membrane protein EP-A-0372501), synthetic peptides (EP-A-0378881, EP-A-0427347), heat shock proteins (WO93/17712, WO94/03208) pertussis proteins (WO98/58668, EP-A-0471177) protein D from *H.influenzae* (WO00/56360) cytokines (WO91/01146), lymphokines, hormones, growth factors, toxin A or B from *C.difficile* (WO00/61761) iron-uptake proteins WO01/72337) *etc.* Where a mixture comprises capsular saccharides from both serogroups A and C, it may be preferred that the ratio (w/w) of MenA saccharide:MenC saccharide is greater than 1 (*e.g.* 2:1, 3:1, 4:1, 5:1, 10:1 or higher). Different saccharides can be conjugated to the same or

different type of carrier protein. Any suitable conjugation reaction can be used, with any suitable linker where necessary.

Toxic protein antigens may be detoxified where necessary *e.g.* detoxification of pertussis toxin by chemical and/or genetic means. Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens.

Antigens in the composition will typically be present at a concentration of at least 1μg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen. As an alternative to using protein antigens in the composition of the invention, nucleic acid encoding the antigen may be used Robinson & Torres (1997) *Seminars in Immunology* 9:271-283; Donnelly *et al.* (1997) *Annu Rev Immunol* 15:617-648; Scott-Taylor & Dalgleish (2000) *Expert Opin Investig Drugs* 9:471-480; Apostolopoulos & Plebanski (2000) *Curr Opin Mol Ther* 2:441-447; Ilan (1999) *Curr Opin Mol Ther* 1:116-120; Dubensky *et al.* (2000) *Mol Med* 6:723-732; Robinson & Pertmer (2000) *Adv Virus Res* 55:1-74; Donnelly *et al.* (2000) *Am J Respir Crit Care Med* 162(4 Pt 2):S190-193 and Davis (1999) *Mt. Sinai J. Med.* 66:84-90. Protein components of the compositions of the invention may thus be replaced by nucleic acid (preferably DNA *e.g.* in the form of a plasmid) that encodes the protein.

DISEASE STATES

The compositions of the present invention may be used to prevent and/or treat disorders such as but not limited to: pneumonia, cardiovascular diseases, atherosclerosis, bronchitis, pharyngitis, laryngitis, sinusitis, obstructive lung diseases, asthma, chronic obstructive pulmonary disease, reactive arthritis, otitis media, abdominal aortic aneurysm, erythema nodosum, Reiter syndrome, sarcoidosis, Alzheimer's disease, multiple sclerosis, lymphogranuloma venereum, ocular trachoma, pelvic inflammatory disease, inclusion conjunctivitis, genital trachoma, infant pneumonitis, incipient trachoma, keratitis, papillary hypertrophy, corneal infiltration, vulvovaginitis, mucopurulent rhinitis, salpingitis, cervicitis, cervical follicles, prostatitis, proctitis, urethritis, lymphogranule inguinale, climatic bubo, tropical bubo, and/oresthiomene.

FORMULATIONS

*Chlamydia*l infections affect various areas of the body and so the compositions of the invention may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared (*e.g.* a lyophilised composition). The composition may be prepared for topical administration *e.g.* as an ointment, cream or powder. The composition may be prepared for oral administration *e.g.* as a tablet or capsule, as a spray, or as a syrup (optionally flavoured). The composition may be prepared for pulmonary administration *e.g.* as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration *e.g.* as drops. The composition may be in kit form, designed such that a combined composition is reconstituted just prior to

administration to a patient. Such kits may comprise one or more antigens in liquid form and one or more lyophilised antigens.

Further components of the composition

The composition of the invention will typically, in addition to the components mentioned above, comprise one or more 'pharmaceutically acceptable carriers', which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The vaccines may also contain diluents, such as water, saline, glycerol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. A thorough discussion of pharmaceutically acceptable excipients is available in Gennaro (2000) *Remington: The Science and Practice of Pharmacy.* 20th ed., ISBN: 0683306472.

The biological molecules of the present invention be formulated into a pharmaceutical composition or an immunotherapeutic composition or a vaccine composition. Such formulations comprise biological molecules combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampoules or in multi-dose containers containing a preservative. Formulations include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (for eg, a powder or granules) form for reconstitution with a suitable vehicle (e. g., sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono-or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

KITS

Also included in the invention is a kit for enhancing a CMI response to the biological molecules of the present invention. Such a kit may comprise an antigenic composition

or nucleotide sequence encoding same. The kit may also include an adjuvant, preferably a genetic adjuvant is administered with or as part of the biological molecule and instructions for administering the biological molecule. Other preferred components of the kit include an applicator for administering the biological molecule. As used herein, the term "applicator" refers to any device including but not limited to a hypodermic syringe, gene gun, particle acceleration device, nebulizer, dropper, bronchoscope, suppository, impregnated or coated vaginally-insertable material such as a tampon, douche preparation, solution for vaginal irrigation, retention enema preparation, suppository, or solution for rectal or colonic irrigation for applying the NOI either systemically or mucosally or transdermally to the host subject.

The invention also provides for a kit comprising comprising a combination of *Chlamydia pneumoniae* antigens. The combination of *Chlamydia pneumoniae* antigens may be one or more of the immunogenic compositions of the invention. The kit may further include a second component comprising one or more of the following: instructions, syringe or other delivery device, adjuvant, or pharmaceutically acceptable formulating solution. The invention also provides a delivery device pre-filled with the immunogenic compositions of the invention.

EXAMPLES

The following invention will now be further described only by way of example in which reference is made to the following Figures. The following examples are presented only to illustrate the present invention and to assist one of ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the invention. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

Figure 1A. Assay of *in vitro* neutralization of *C.pneumoniae* infectivity for LLC-MK2 cells by polyclonal mouse antisera to recombinant *Chlamydia*l proteins. Results are shown as reduction in the number of inclusions obtained when monolayers were infected with antiserum-treated infectious EBs, as compared to inclusion numbers given by untreated EBs. Percent reduction values are plotted against the reciprocal of the corresponding serum dilution. For each dilution inclusion counts were corrected for background inhibition of infectivity observed with the corresponding dilution of the pre-immune serum. The figure shows results obtained with serial dilutions of antibodies raised against a 'neutralizing' antigen (♥), a 'non-neutralizing' FACS- positive antigen (ν), and against the GST polypeptide, used in the fusion constructs, alone (σ).

Figure 1B shows serum titres giving 50% neutralization of infectivity for the 10 *C.pneumoniae* recombinant antigens described in the text. Each titer was assessed in 3 separate experiments (SEM values shown).

Figure 2 shows immunoblot analysis of two dimensional electrophoretic maps of *C.pneumoniae* EBs using the imune sera described in the text. Immunoblots were obtained from either of two EB gels (panels A and B at the top) covering different pH intervals, according to which of the two allowed the best detection of a given antigen. The arrows in the HtrA immunoblot show which of the signals had a corresponding stained spot in the gel (arrows in panel A) which was subjected to MALDI-TOF

identification. The two patterns in the HtrA blot are both suggestive of typical electrophoretic 'trains' composed of single charge variants of the same protein.

Figure 3 shows mean numbers of *C.pneumoniae* IFU recovered from equivalent spleen samples from immunized and mock-immunized hamsters following a systemic challenge. Standard deviation values are shown above the bars. Antigens which induced significant protection are highlighted with an asterisk above the corresponding bar. All antigens were were delivered in Freund's adjuvant. n.i. = non immunized controls

Figure 4 shows flow cytometric analysis of splenocytes from DNA-immunized HLA-A2 transgenic and non transgenic mice. Groups of 4 mice were immunized 3 times i.m. with 50μg of plasmid DNA expressing *C. pneumoniae* Low Calcium Response Protein H. IFN-γ production from splenocytes was monitored following either a 6h (ex-vivo) or a 6 day (restimulated) pulse with peptide CH-6 (10μg/ml). Equal numbers of gated live lymphocyte cells were acquired with a LSRII FACS System (Becton Dickinson) and percentages of IFN-γ producing $CD8^+$ T cells were calculated using DIVA Software (Becton Dickinson).

Figure 5 shows a flow cytometric analysis of splenocytes from transgenic and non transgenic mice infected with *C. pneumoniae* EBs. (A) HLA-A2 transgenic mice were intranasally infected twice with $5x10^5$ *C. pneumoniae* FB/96 EBs and splenocytes were stimulated for 6 days in the presence of relevant peptides before determining IFN-γ production by $CD8^+$ T cells as described in the legend of Figure 4. (B) HLA-A2 transgenic and non transgenic mice were infected together with the same EBs preparation and $CD8^+$ T cells were subjected to FACS analysis as reported in (A).

Table I shows a summary of data and properties of the *C.pneumoniae* antigens described in the text. The neutralization titer is reported is as the reciprocal of the antiserum dilution causing a 50% reduction in the number of inclusions in the *in vitro* infectivity assay. For the hamster model data the statistical significance of the results was evaluated by a two-tailed Student's t-test: significant data ($p \leq 0.05$) are highlighted with an asterisk. ND = not detected.

Table 2 shows results from hamster mouse model studies for hypothetical proteins.

Table 3 shows expressed genes of CPn EB selected by microarray.

Table 4 shows *C. pneumoniae* selected peptides: protein sources and HLA-A2 stabilization assay.

Table 5 shows ELISPOT assay with CD8+ T cells from DNA immunised HLA-A2 transgenic mice.

Table 6 shows IFN-γ production from splenocytes of DNA immunized HLA-A2 transgenic and non transgenic mice.

METHODS AND MATERIALS (Examples 1-4) (see Reference Section 1)

C.pneumoniae EB purification

C.pneumoniae FB/96, a clinical isolate obtained from a patient with pneumonia at the Sant'Orsola Polyclinic, Bologna, Italy, was grown in LLC-MK2 cells seeded in individual wells of a six-well plastic plate (7). Cells were harvested 72 hr after infection with a sterile rubber, sonically disrupted and the elementary bodies (EB) purified by gradient centrifugation as described (26). Purified *Chlamydiae* were resuspended in sucrose-phosphate-glutamic acid (SPG) transport buffer, and stored in 0.5 ml aliquots, at –80°C until used. When required, prior to storage, EB infectivity was heat-inactivated by 3 hour incubation at 56°C.

Expression and purification of recombinant proteins

Open reading frames (ORFs), selected from the *C. pneumoniae* CWL029 genome sequence (16), were PCR-cloned into plasmid expression vectors and purified from *E.coli* cultures, essentially as previously described (25). Recombinant *Chlamydial* proteins were obtained as GST fusion proteins by using pGEX-KG derived vectors (12) in *E. coli* BL21 (Novagen). PCR primers were designed so as to amplify genes without the N-terminal signal peptide coding sequence. When a signal peptide or processing site was not clearly predictable, the ORF sequence was cloned as annotated by Kalman and coworkers (16). Recombinant *E.coli* cells were grown in LB medium (500 ml), containing 100 μg/ml Ampicillin, and grown at 37°C until OD_{600} = 0.5 , and then induced with 1 mM IPTG. Cells were collected by centrifugation 3 hr after induction and broken in a French Press (SLM Aminco, Rochester, NY). After centrifugation at 30.000 g, the supernatants were loaded onto Glutathione Sepharose 4B columns (Amersham Pharmacia Biotech) and column bound proteins were eluted with 50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0. Protein concentrations in the samples were determined using the Bradford method.

Preparation of mouse antisera

Groups of four 5/6-week old CD1 female mice (Charles River, Como, Italy) were immunized intraperitoneally at day 1 with 20ug of protein in Complete Freund's adjuvant (CFA) and boosted at day 15 and 28 with 20ug of recombinant protein in Incomplete Freund's adjuvant (IFA). Pre-immune and immune sera were prepared from blood samples collected on days 0, 27 and 42. In order to reduce the amount of antibodies possibly elicited by contaminating *E. coli* antigens, the immune sera were incubated overnight at 4°C with nitrocellulose strips adsorbed with a total protein extract from *E. coli* BL21.

Flow cytometry assays

Analyses were performed essentially as previously described (25). Gradient purified, heat-inactivated EBs (2x105 cells) from *C.pneumoniae* FB/9, resuspended in phosphate-saline buffer (PBS), 0.1% bovine serum albumin (BSA), were incubated for 30 min. at 4°C with the specific mouse antisera (standard dilution 1:400). After centrifugation and washing with 200 μl of PBS-0.1% BSA, the samples were incubated for 30 minutes at 4°C with Goat Anti-Mouse IgG, F(ab)'2-specific, conjugated with R-Phycoerythrin (Jackson Immunoresearch Laboratories Inc.). The samples were washed with PBS-0.1%BSA, resuspended in 150 μl of PBS-0.1%BSA and analysed by Flow Cytometry using a FACSCalibur apparatus (Becton Dickinson, Mountain View, CA). Control samples were similarly prepared. Positive control antibodies were: i), a commercial anti-*C.pneumoniae* specific monoclonal antibody

(Argene Biosoft, Varilhes, France) and, ii), a mouse polyclonal serum prepared by immunizing mice with gradient purified *C.pneumoniae* EBs. Background control sera were obtained from mice immunized with the purified GST peptide used in the fusion constructs (GST-fusions control). FACS data were analysed using the Cell Quest Software (Becton Dickinson, Mountain View, CA). The shift between the background control histogram and the immune serum testing histogram was taken as a measure of antibody binding to the EB cell surface. The Kolmorov-Smirnov (K-S) two-sample test (44) was performed on the two overlapped histograms. The D/s(n) values (an index of dissimilarity between the two curves) are reported as "K-S score" in Table 1.

2D Western Blot analysis of immune sera, and Mass Spectrometry

Gradient purified *C. pneumoniae* EBs were washed with 5 mM Tris-HCl pH 7.5, 0.1 mM EDTA, 10% glycerol, centrifuged 15 min. at 13 000 x g and pellets were resuspended in reswelling solution (7 M urea, 2 M thiourea, 2% (w/v) CHAPS, (2%w/v) ASB14, 2% (v/v) IPG buffer pH 3–10 NL, or pH 4-7, 2 mM TBP, 65 mM DTT). Protein samples (200 or 20 µg of protein for Coomassie Blue stained reference gels, or gels to be processed for immunoblotting, respectively) were adsorbed overnight on Immobiline DryStrips (7 cm, pH 3-10 NL, or pH 4-7). Electrofocusing was performed in an IPGphor Isoelectric Focusing Unit (Amersham Biosciences, Uppsala, Sweden). The focused strips were equilibrated as described (15) and loaded on linear 9-16.5 % acrylamide gradients (7x 4 cm, 1.5 mm thick), for SDS-PAGE separation in a Mini Protean III Cell (Bio-Rad, Hercules, CA). Gels were stained with colloidal Coomassie Blue (Novex, San Diego, CA) (4) and the protein maps so obtained were scanned with a Personal Densitometer SI (Molecular Dynamics) at 12 bits and 50 mm per pixel.

For Western Blot analyses, the proteins separated in the 2DE maps were transferred onto nitrocellulose membranes, overnight at 30 Volts, using a Protean III apparatus (BioRad, Hercules, CA). Membranes were stained with a 0.05% (w/v) CPTS (Copper(II) phthalocyanine-3,4',4'',4'''-tetrasulfonic acid tetrasodium salt) in 12 mM HCl, and marked peripherally with 8 India-ink dots to provide anchors for subsequent image superimposition and matching. After scanning and image acquisition, the membranes were destained with 0,5 M NaHCO3, incubated with the mouse sera to be analyzed (either pre-immune or specific immune sera, diluted 1:1000), and then with a peroxidase-conjugated anti-mouse antibody (Amersham Biosciences, Uppsala, Sweden). After washing with PBS, 0.1% Tween-20, blots were developed using the Opti-4CN Substrate Kit (Biorad, Hercules, CA), and the images of the immunostained blots again acquired as above. Images were analysed with the computer program Image Master 2D Elite, version 4.01 (Amersham Biosciences, Uppsala, Sweden). Superimposition and matches between Western-blot membranes and Coomassie stained gels were performed as follow. First, the CPTS-stained membrane image and the immunostained blot image were superimposed using the peripheral dot marks. Then, the sum image so obtained was superimposed to the Coomassie stained protein map using the CPTS stained CPn proteins as anchors. The areas on the Coommassie stained map corresponding to immunostained spots on the blot were excised from the preparative gel for protein identification. Protein sample were dried in a vacuum centrifuge, and in-gel digested, for 2h at 37°C, with an excess of porcine Trypsin (Promega, Madison, WI), in 100 mM ammonium bicarbonate. Tryptic peptides were desalted and concentrated using Zip-Tip (Millipore, Bedford, MA). Peptides were directly eluted and loaded onto a SCOUT 384 Anchor Chip multiprobe plate (400 µm,

Bruker Daltonics, Bremen, Germany) with a solution of 2-5 dihydroxybenzoic acid (5g/l), in 50% acetonitrile, 0.1% trifluoroacetic acid. Spectra were acquired on a Bruker Biflex III matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) apparatus. Resulting values for monoisotopic peaks were used for database searches using the Mascot software (32), as available at the website http://www.matrixscience.com/.

In vitro neutralization assays

In vitro neutralization assays were performed on LLC-MK2 (Rhesus monkey kidney) epithelial cell cultures. Serial four-fold dilutions of mouse immune and corresponding preimmune sera were prepared in sucrose–phosphate–glutamic acid buffer (SPG). Mouse polyclonal sera to whole EBs were used as positive control of neutralization, whereas SPG buffer alone was used as negative control of neutralization (control of infection). Purified infectious EBs from the *C.pneumoniae* FB/96 were diluted in SPG buffer to contain $2.5x10^7$ IFU/ml, and 10ul of EBs suspension were added to each serum dilution in a final volume of 100ul. Antibody-EB interaction was allowed to proceed for 30 min at 37°C on a slowly rocking platform. The 100ul of reaction mix of each sample was used to inoculate PBS-washed LLC-MK2 confluent monolayers (in triplicate for each serum dilution), in a 24-well tissue culture plate, and centrifuged at 805 x g for 1 hour at 37°C. After centrifugation Eagle's minimal essential medium containing Earle's salts, 20% fetal bovine serum and 1ug/ml cycloheximide was added. Infected cultures were incubated at 37°C in 5%CO_2 for 72 hours. The monolayers were fixed with methanol and the *Chlamydia*l inclusions were detected by staining with mouse anti-*Chlamydia* fluorescein-conjugated monoclonal antibody (Merifluor *Chlamydia*, Meridian Diagnostics, Inc.) and quantified by counting 10 fields per well at a magnification of 40X. The inhibition of infectivity due to EBs interaction with the immune sera was calculated as percentage reduction in mean IFU number as compared to the SPG (buffer only)/EBs control. In this calculation the IFU counts obtained with immune sera were corrected for background inhibition of infection due to the corresponding pre-immune mouse serum. According to common practice, the sera were considered as "neutralizing" if they could cause a 50% or greater reduction in infectivity. The corresponding neutralizing titer was defined as the serum dilution at which a 50% reduction of infectivity was observed. Experimental variability was evaluated by calculating the standard error of measurement (SEM), from three titration experiments for each recombinant antigen, as shown in Fig.1B.

In vivo screening

In vivo evaluation was performed using a hamster model of systemic infection, as recently described (34). Essentially, adult (10-11 week old) Syrian hamsters (Morini, S. Polo D'Enza, Italy), previously immunized with the recombinant vaccine candidates were challenged systemically with infectious Cpn elementary bodies (EB). Protection was assessed by determining the levels of viable EB recovered from the spleen, as compared to non-immunized animals. Statistical significance of the results was evaluated by a two-tailed Student's t-test.

Groups of 8 hamsters were immunized subcutaneously with recombinant antigens, or only injected with buffer for the control group, at days 0, 7, and 21. For each immunization 20 ug protein 1:1 diluted with Freund's complete adjuvant (first dose) and Freund's incomplete adjuvant (booster doses) was injected. At day 35 post-

infection the hamsters were anaesthetised with Ketamine and inoculated intraperitoneally and intranasally with 0.1 ml of *C.pneumoniae* EB suspension ($1.0x10^8$) at each site. Animals were sacrificed seven days after infection. The spleen was weighed, and homogenized in a mortar to obtain a 10% (wt/vol) suspension in cold SPG buffer. Tissue suspensions were centrifuged at 300 x g for 10 min at 4°C to remove coarse debris. The clarified homogenates (0.2 ml) were inoculated in duplicate onto LLC-MK2 cells seeded in plastic individual well of a 24 well plate, incubated at 37°C for 72 h and fixed in acetone before detection and counting of numbers of *Chlamydia*l inclusions per well by immunofluorescence microscopy. The protocol was approved by the ethical committee of the University of Bologna.

Example 1 (in vitro studies)

Screening antisera for in vitro neutralizing properties

Following a genome-wide screening for proteins likely to be localized on the cell surface of *C. pneumoniae*, we recently reported (25) that antisera to 53 recombinant *Chlamydia*l antigens were capable to bind in a FACS assay, the surface of *Chlamydia*l cells. In order to check whether some of the FACS positive antigens were capable of interfering with EB *in vitro* infectivity, we raised mouse antisera against the recombinant FACS positive antigens and assessed the effect of each antiserum on the infectivity of purified EBs with respect to monolayers of LLC-MK2 cells. Infectious EB were first incubated with the antiserum and then used to infect cell monolayers in 24-well multititer plates. In parallel, control samples were similarly processed in which the EBs were: i), either treated with buffer only, or, ii), treated with the same dilutions of the corresponding preimmune mouse sera.

Results I

Using this assay, 10 sera have so far proved to effectively neutralize *in vitro* infectivity to an extent greater than 50%, a property that common practice qualifies such antigens as "neutralising" (Figure 1). These 10 sera were obtained by mouse immunization with recombinant proteins derived from the following *C.pneumoniae* genes:

- *pmp10* and *pmp2*, encoding two members of the heterogeneous *Chlamydia*l PMP family of polymorphic membrane proteins;
- *artJ*, encoding a putative extracellular solute (possibly Arginine) binding protein of an aminoacid transport system;
- *eno*, encoding a protein homologous to bacterial enolases, glycolytic enzymes which can be found also on the bacterial surface;
- *htrA*, encoding a putative chaperone with heat-shock inducible protease activity;
- the Cpn0301 "hypothetical" gene, encoding a protein homologous to the ompH family of bacterial proteins, some members of which have been shown to be chaperones involved in outer membrane biosynthesis;
- two Cpn-specific "hypothetical" genes Cpn0795 and Cpn0042;
- *omc*A encoding a 7-9 kDa protein annotated as an outer membrane protein; and
- *ato*S a putative sensor member of a transport system.

As shown in Figure 1 and summarized in Table I, OmpH, enolase and Cpn0795 appeared to induce the highest neutralizing sera, with titers above 400. By contrast, Pmp2, ArtJ and Cpn0042 induced titers equal or less than 100, while the remaining 4 antigens, Pmp10, HtrA, AtoS and OmcA showed intermediate titers.

Example 2 (in vivo studies)

Evaluation of antisera specificity by 2D immunoblot analysis of Cpn protein extracts

In order to investigate if the neutralizing activity observed in the *in vitro* infection of LLC-MK2 monolayers was actually due to the binding of the antibodies to the selected *C.pneumoniae* proteins, rather than to possible cross-rections with other antigens, we assessed the specificity of the antisera by immunoblot analysis of two dimensional electrophoretic maps of EB proteins.

In particular, this analysis was carried out on six antigens (Pmp2, Pmp10, Eno, ArtJ, HtrA and OmpH-like) known to be visible in the 2D maps of EB total proteins (Montigiani *et al.*, 2002 Infection and Immunity 70: 368-379). Total EB proteins were resolved by 2D-electrophoresis using two different pH intervals (pH 3-10 non linear, and pH 4-7, respectively) since previous experiments had shown that some of the proteins under study were better detected using one rather that the other of the above pH intervals. For each pH interval four gels were run in parallel. One gel was stained with Coomassie Blue to visualize the protein spots, while the other gels were blotted on nitrocellulose filters and stained with one of the selected sera at 1000-fold dilution. Subsequently, the images of the immunostained blots (Fig.2, panels c to h) were superimposed to the corresponding Coomassie Blue-stained gel to identify the spots which had reacted with a given antiserum. The matching protein spots were excised and processed for peptide identification by MALDI-TOF analysis.

Results 2

In all six maps the immunoreactive protein species in the excised gel area were found to contain peptides from the expected *Chlamydia*l protein. Even when the serum reacted with more than one electrophoretic protein species, the mass spectra of all spots which could be detected in the Coomassie Blue stains 2DE map were always consistent with the same polypeptide being present as multiple electrophoretic species.

Interestingly, the immunoblot obtained with the HtrA antiserum showed two sets of 4 spots arranged as two typical electrophoretic 'trains' at two different molecular weights. On the Coomassie Blue stained gel it was possible to identify 4 corresponding spots, 3 in the upper train and 1 in the lower Mw set. MS analysis identified all of them as products of Cpn HtrA gene. Interestingly the lower Mwt species missed 3 N-terminal tryptic peptides, detected in the higher Mw spot series, and mapping within the first 100 aa of the ORF. These results suggest that HtrA was present in the EB protein sample both as a full *htr*A product, and as a discrete species missing a short N-terminal peptide, possibly as a result of some post-translational processing.

Discussion of Results 2

In the analysis of data which are based on polyclonal antibody reactivity one should consider that cross-reactions due to epitope mimickry are always difficult to exclude. The problem of antisera specificity was addressed in this work by 2D immunoblotting and identification of the reacting electrophoretic species by mass spectrometry analysis. This approach was possible for 6 of the 10 antisera, i.e. those corresponding to proteins previously identified by mass spectrometry (MALDI-TOF) analysis on 2D electrophoretic maps of *C.pneumoniae* EB proteins (25, 42) (Table 1, and Figure 2).

The probability of fortuitous cross-reactions between unrelated *Chlamydial* protein species was minimized by the results of the immunoblotting analyses which showed that out of ca 300 protein spots in a map, all those reacting with the tested antisera were consistent with the expected antiserum specificity. Obviously, since during 2-D electrophoresis conformational epitopes are generally lost, structure-dependent cross-reactions cannot be ruled out in this type of analysis.

Example 3

In vivo evaluation of the in vitro neutralizing antigens in a hamster model of systemic infection

We have recently described a new hamster model of systemic *Chlamydia pneumoniae* infection in which replicating *Chlamydia* disseminate through macrophages and accumulate in the spleen (34). We therefore asked the question whether the *in vitro* neutralizing antigens we identified would also have protective activity *in vivo* using this model. To this aim, the 10 *in vitro* neutralizing recombinant antigens were used to immunize 8 hamsters with 3 subcutaneous injections over a three-week period, and challenged with $2x10^8$ Cpn EBs two weeks later. Spleen infection was assessed 7 days after challenge. The difference between the mean number of infectious *Chlamydiae* recovered from control animals and the mean number of *Chlamydiae* recovered from animals immunized with the recombinant *Chlamydial* antigens, was taken as a measure of protection specifically induced by the putative vaccine candidate.

Results 3

The results of spleen protection observed for the various antigens in repeated experiments are shown in Figure 3 and reported as percentage values in Table 1. Six out of ten antigens, Pmp2, Pmp10, Enolase, the OmpH-like protein, and the products of the *C.pneumoniae*-specific genes Cpn0759 and Cpn0042, showed a statistically significant protective activity, with a reduction in IFU recovered from the spleens of immunized animals higher than 80% with respect to mock-immunized controls.

A limit of the hamster model is that, because of the absence of immunological reagents, the relative contribution of humoral and cell-mediated immunity cannot be assessed. However, we asked the question whether recombinant antigens could elicit also in the hamster neutralizing antibodies with sufficiently high titers. Therefore we tested the sera from hamsters immunized with Pmp2 and enolase, two of the most protective antigens, in the *in vitro* neutralization assay. Both antigens had a neutralizing titer of approximately 100 (data not shown).

Summary of Results 3

In conclusion, a considerable proportion (60%) of the *in vitro* neutralizing antigens were also protective in the hamster *in vivo* model and our data suggest that antibody-mediated neutralization could play a role at least in this model of systemic infection.

Discussion of Results 3

Beside assaying the *in vitro* neutralization properties of the selected subset of 10 FACS-positive antigens, we also assessed the performance of these antigens in protecting against *C. pneumoniae* infection in an animal model of systemic infection recently described in the hamster (34). This evaluation addressed the capability that the recombinant antigens would have of inducing a protective response against naturally replicating *Chlamydiae* (rather than EB's purified from *in vitro* cultures

grown under artificial conditions) and in the context of a systemic infection. In fact the hamster model we used, while it does not model the typical respiratory infection considered to be the predominant route by which *C. pneumoniae* infects humans, it nevertheless simulates a situation of systemic invasion which could be preliminary to the establishment of *C. pneumoniae* chronic infection considered by several authors as being associated to the development or progression of cardiovascular disease, and other chronic degenerative diseases. Notably, a limit of any hamster model is the current lack of hamster-specific immunological reagents which would allow the analysis of cell mediated immune responses. However, in the case of systemic infections, by common wisdom, neutralizing antibodies are likely to have a protective action. The finding that 6 of the 10 '*in vitro* neutralizing' antigens had also a >80% protective action *in vivo*, and that a measurable neutralizing activity was also found in the sera of immunized hamsters, suggests that a specific antibody mediated immunity could at least partially contribute to the animal protection here described.

Example 4

Two 'hypothetical'proteins 6784 and 6814 (encoded by the ORFs Cpn0498 and Cpn0525) yielded FACS-positive sera which, however, were not able to neutralize host cell infection *in vitro*. However, these antigens performed remarkably well in the hamster-spleen test.

Table 2

Gene/ORF ID in CWL029	Protein ID	Recombinant Fusion Type	Annotation	Reciprocal of 50% neutralisation titre	% Protection in the hamster spleen test (ref 34)
Cpn0498	4376784	GST	Hypothetical protein	0	94
CPn0525	4376814	GST	Hypothetical protein (similarity to CT398)	0	97
CPn0324		HIS	Low Calcium Response Element (LcrE)		Completely protected 8 of 16 animals and reduced the infectivity titres of the eight positive animals

Discussion of Results 4

Interestingly, whilst antiserum against CPn0525 gave negative *in vitro* results (ie no neutralising activity), the CPn0525 protein gave 97 per cent protection from spleen infection in an *in vivo* hamster immunisation assay (ie a positive *in vivo* result). Likewise, whilst antiserum against Cpn0498 gave negative *in vitro* results (ie no neutralising activity), the CPn0498 protein gave 94 per cent protection from spleen infection in an *in vivo* hamster immunisation assay. Thus a positive signal obtained in the FACS assay does not guarantee a corresponding positive *in vitro* neutralization activity and conversely a negative neutralization activity does not mean that a positive *in vivo* result can be excluded.

General Discussion of Results 1-4

Strategy for identification of Chlamydia pneumoniae antigens of interest

Our strategy was based on the following experimental steps: 1) analysis of *Chlamydia* genome sequence to select putative membrane-associated antigens, 2) cloning, expression and purification selected antigens, 3) preparation of antigen-specific sera by mouse immunization with the purified antigens, 4) FACS analysis of *Chlamydia* EBs using the mouse sera to identified surface-exposed antigens, 5) "*in vitro* neutralization" assay to test whether antibodies elicited by a given antigen can interfere with the process of eukaryotic cell infection, and 6) use of appropriate animal model to test the capacity of selected antigens to confer protection against *Chlamydia* challenge.

As recently described by Montigiani *et al* ((2002) Infection and Immunity 70: 368-379) from the initial screening of the *C.pneumoniae* genome, a panel of mouse sera was prepared against over 170 recombinant His-tagged or GST-fusion proteins encoded by genes or "open reading frames" somehow predicted to be peripherally located in the *Chlamydial* cell. When these antibodies were tested in a FACS assay for their ability to bind the surface of purified *C.pneumoniae* EBs, a list of 53 "FACS-positive" sera was obtained. The corresponding putative surface antigens were then further assessed for their capability of inducing neutralizing antibodies. This part of the work involved testing which of the sera contained antibodies capable of interfering with the process of *in vitro* infection of epithelial cell cultures. In the *in vitro* "neutralization" assay purified infectious EBs are incubated with progressive dilutions of the immune sera and, in parallel, dilutions of the corresponding pre-immune sera, and of sera against non *Chlamydia* control antigens.

Cell cultures are infected in the presence of cycloheximide, which inhibits host cell protein synthesis and favours *Chlamydial* intracellular growth with the consequent formation of typical cytoplasmic inclusions which can be stained with *Chlamydia* specific fluorescence-labeled monoclonal antibodies and counted with an UV light microscope. Working with appropriate pathogen-to-host cell ratios, it can be reasonably assumed that the number of detected cytoplasmic inclusion is proportional to the number of infectious *Chlamydiae* in the original sample. So a reduction in inclusion numbers caused by the presence of an antigen-specific antiserum, as compared to the numbers obtained with control sera, gives a measure of the capability of a given antigen to elicit antibodies which can inhibit some stage of the *Chlamydial* infection process. According to common convention, an anti-serum is labelled as 'neutralizing' when the reduction of infectivity is equal or greater than 50%, and the

serum dilution yielding a 50% reduction in infectivity is referred to as the 50% end-point neutralization titer.

Some of the results obtained by screening the panel of recombinant antigens with the *C.pneumoniae in vitro* neutralization assay confirm that some of the listed antigens, like the members of the family of heterogeneous polymorphic membrane proteins (PMP), which, on the basis of published literature data, could be reasonably expected to be surface-exposed and possibly induce neutralizing antibodies. However, there are also proteins which could be considered so far only hypothetical, and proteins which just on the basis of their current functional annotation could not be at all expected to be found on the bacterial surface. Using an *in vitro* neutralising assay, it was found that sera to 10 CPn antigens have so far proved to effectively neutralize *in vitro* infectivity to an extent greater than 50%, a property that common practice qualifies such antigens as "neutralising" (Figure 1). These 10 sera were obtained by mouse immunization with recombinant proteins derived from the *C.pneumoniae* genes listed below.

Using a recently described *in vivo* model of systemic infection (hamster model), hamsters immunised with 6 of the *in vitro* neutralising antigens, when challenged with CPn EBs, showed a greater than 80% reduction of spleen infection as compared with non-immunised controls.

Characterisation of 10 CPn proteins

The proteins identified by the present work can be divided in 3 groups:

- proteins which have an annotation compatible with (could be reasonably expected to have) an expected/predicted exposure on the *Chlamydia*l cell surface and with the possibility that antibodies binding to them may actually interfere with host cell attachment and entry (ie proteins which could possibly induce neutralising antibodies)
- proteins which by homology with other gram-negative bacteria could be expected to have a periplasmic exposure (ie would not be expected at all to be found on the bacterial cell surface); and
- proteins which are still labelled as 'hypothetical' (ie cellular location and/or cellular function not known)

Group 1

(Pmp proteins (pmp2 and pmp10), OmcA and OmpH)

Pmp proteins (pmp2 and pmp10)

The first group includes the 2 polymorphic outer membrane proteins (Pmp's) Pmp2 and Pmp10 (10, 11, 14, 30), the outer membrane protein OmpH-like, and OmcA, which is annotated (*Chlamydia* Genome Project at http://*Chlamydia*-www.berkeley.edu:4231/) as "predicted 9-kD cysteine-rich, outer membrane protein, lipoprotein". The Pmp family of *Chlamydia*-specific proteins is generally thought to comprise probable pathogenicity factors, with an autonomous secretion capacity (autotransporters), important for adhesion to host cells and are generally considered as promising vaccine candidates. However, apart from very recent unpublished results on Pmp21, this is the first time that antisera to recombinant Pmp's are reported to have neutralizing properties.

OmcA

OmcA is the product of a gene co-transcribed in the same operon with the 60 kDa OmcB cystein-rich protein which is a major structural component of the *Chlamydia*l outer membrane and a major immunogen in human *C. trachomatis* infections. OmcB and OmcA are likely to interact in some as yet unknown outer membrane structure, so it is possible that antibodies to OmcA can interfere with EB infectivity.

OmpH

Finally, the *Chlamydia*l OmpH is probably a member of the OmpH (Skp) family of proteins which have been reported to have chaperonin activities in other bacteria very important for the correct biosynthesis of the outer membrane. These proteins appear to cooperate in this task with HtrA (see below). In fact, in *E.coli* single KO mutants of either OmpH (Skp) or HtrA (DegP) are still viable, but double mutants do not grow (37). It should be pointed out that even if the amino acid sequences of the ompH-like proteins of *Chlamydia* (all *C.pneumoniae* and *C.trachomatis* or *C.caviae* variants) line-up very well with the rest of the bacterial OmpH proteins, they are the only ones to be acidic, whereas the rest of the family comprises mostly very basic proteins (including some with histone like behaviour, at least *in vitro*). One could speculate that if the chaperone activity is maintained also in the ompH like *Chlamydia*l proteins, this may be related to some *Chlamydia*l peculiarity.

Second Group of Selected Proteins
(ArtJ, AtoS, HtrA and Enolase)

The second group, which represents a somehow surprising finding, includes ArtJ, AtoS, HtrA and Enolase. If the current annotation (justified by analogy with homologous genes in other bacteria) is correct, all these proteins would be expected to have a periplasmic location in gram-negative bacteria. and to be surface-exposed only in a gram-positive bacterium. It is possible that owing to their atypical life cycle, requiring an efficient passage from a dormant spore-like status (the EB) to an active form needing to adapt quickly to host-cell responses to invasion, *Chlamydia*e in fact display some sensors directly on the outer surface of their infectious form.

ArtJ

In the case ArtJ – for which we have data supporting both antigen expression and serum specificity – the hypothesis of an atypical situation peculiar to *Chlamydia* is supported by the anomalous gene set-up resulting from the present analysis of the *Chlamydia* genomes. ArtJ is so annotated by analogy with the ART transport systems of *E.coli* which has 5 genes organized in two operons (24) : artPIQM and artJ which are responsible for the arginine transport. In Cpn however the artPIQM genes are absent and therefore it appears that *Chlamydia*l ArtJ operates in a molecular context which is different from its *E.coli* model and must be peculiar to this species.

HtrA

HtrA (DegP), which in other bacteria has a complex hexameric structure, has been described as having multiple functions (3, 5, 18, 19, 27, 38) : a chaperonin assisting a correct outer membrane biogenesis, inducible protease for the elimination of misfolded membrane proteins, and also a sensor of 'stress' conditions. In *Chlamydia* none of these properties has been demonstrated yet, however we find that in purified EB HtrA is present in two forms one of which appears to be processed by being deprived of the N-terminal fragment. This fragment, if aligned with the homologous

HtrA sequence from Thermologa maritima (18), would comprise a predicted loop acting as a structural lid controlling the access to the protease active. So it appears tempting to speculate that HtrA could have a similar protease activity and the two forms identified on the 2-D map represent the active and inactive species. Interestingly, the *C. trachomatis* HtrA ortholog is recognized by human sera from patients who had a *Chlamydia*l genital infection (35), and a similarly HtrA is one of the antigens in the immunoproteome of Helicobacter pylori (13). Furthermore the homologue protein in *Haemophilus influenzae* is a protective antigen in both a passive infant rat model of bacteremia and the active chinchilla model of otitis media (23) .

Enolase

Also in the second group of proteins expected to be located elsewhere than the cell surface, is Cpn enolase. This protein aligns with the well known family of conserved glycosylases, which are essentially cytoplasmic enzymes, but in Streptococci enolase has been shown to have also a cell surface location, and extracellular matrix binding properties (1, 28, 29)). Interestingly, Gaston and colleagues (8) also showed that in patients with reactive arthritis induced by *C.trachomatis*, enolase induces specific $CD4^+$ T-cell responses. Furthermore, a clone responding to the enolase *C.trachomatis* ortholog, responded also to *C.pneumoniae* EBs, and, since no proliferative response could be observed using a fungal or a mammalian enolase, the authors of this study concluded that the CD4 T-cell stimulating epitope must be *Chlamydia* specific.

Third Group of Proteins
(unknown cellular location and/or cellular function, Cpn0795, CPn0042)

The third of the 3 groups in which we propose to divide, just for the sake of discussion, the 10 neutralizing antigens above described, comprises two proteins which are still annotated in public *Chlamydial* databases as the hypothetical products of two CPn-specific genes: Cpn0759 and Cpn0042. The Cpn0759 gene is the second gene in a cluster of 6 Cpn-specific hypothetical genes (from Cpn0794 to Cpn0799) immediately upstream of the enolase gene. With the exception of Cpn0759 the products of all the other genes in the cluster share similarities of 30 to 40% over long stretches of amino acids. The Cpn0042 gene encodes a hypothetical protein, with 4 coiled-coil regions, which has been described as a member of a new family of hypervariable outer membrane proteins (33). Interestingly, the hypervariability of these proteins could be due to a strand-slippage mechanism induced by the presence of a poly(C) stretch within the coding region of the corresponding genes, a mechanism already described in the Pmp's family for the *pmp10* gene (30). However, as indicated by their annotation, the function of these proteins is still unknown, and our observations provide the first experimental indication of a possible function related to the *Chlamydial* infection process.

Table 1 of this application demonstrates that Cpn0795 (SEQ ID NO: 6) a Cpn specific hypothetical protein is a FACS positive protein which demonstrates significant immunoprotective activity in a hamster spleen model of Chlamydia pneumoniae infection. We have found evidence to demonstrate that other Cpn proteins in this group of Cpn specific hypothetical proteins have now been found to have a secreted autotransporter function. These proteins, which are absent from *Chlamydia trachomatis* include: gi/4377105 (Cpn0794), gi/4377106 (Cpn0795), gi/4377107 (Cpn0796), gi/4377108 (Cpn0797), gi/4377109 (CPn0798), gi/4377110 (Cpn0799).

Fig. 6 shows an alignment of the proteins in the 7105-7110 protein family. This Alignment shows a new family of proteins expected to constitute a system of antigens probably delivered on the Cpn surface or secreted by a type V (autotransporter) secretion mechanism. This alignment was generated as follows:

Imperfect repeats were identified which allowed the alignment of the genes. Molecular modelling has also demonstrated that the C-terminal ends of 7106 and 7107 can be predicted to fold in a beta-barrel structure which can form a translocation pore for secretion across the outer membrane.

Cpn0794 = 7105 = FACS positive
Cpn0795 = 7106 = FACS positive
Cpn0796 = 7107 = FACS positive
Cpn0797 = 7108 = FACS positive
Cpn0798 = 7109 = No data available
Cpn0799 = 7110 = No data available
(Reference for FACS positive data = Montigiani et al (2002) Infect Immun 70(1) 368-79)
Operon1 = 0794, 0795, 0796 Operon2 = 0797, 0798
Cpn0795 and Cpn0796 have C terminal ends that may form transmembrane pores (see alignment, FIG. 9). CPn0794, Cpn0797, Cpn0798, and Cpn0799 have N-terminal ends indicating that all proteins have N-terminal and C-terminal ends.

Fig. 7 shows alignment of Cpn0794 – Cpn 0799. Proteins encoded by the genes Cpn0794, Cpn0795, Cpn0796, and Cpn0797 have been identified as likely to be exposed on the surface of the chlamydia cell and as possible vaccine candidates. These proteins are shown to be actually expressed by Cpn in vivo (WB data and FACS data). In the case of Cpn0797 we also showed that the level of expression in CPn EBs is high enough to be detected by mass spectrometry analysis on 2DE maps of protein extracts (see Montigiani et al.)

Following these observations, it is seen that the proteins encoded by Cpn0794, Cpn0796 and Cpn0797 proteins can be aligned according to a set of imperfect repeats present within their aminoacid sequences (see FIG. 7) , whereas the putative product of CPn0795 can be mostly aligned to the C-terminal portion of the Cpn0796 protein.

Furthermore, proteins encoded by genes Cpn0798 or Cpn0799 can alse be aligned to the above proteins according to the above mentioned repeated sequence motifs (see FIG. 7).

Overall alignment of the 6 genes demonstrates that the genes encode for a family of functionally-related proteins.

Furthermore, in silico analysis of the protein encoded by Cpn0796, which encompasses the entire alignment of all the proteins in this family demonstrates that a functional precursor with the aminoacid sequence reported below:

SEQ ID NO: 80

MKFMKVLTPWIYRKDLWVTAFLLTAIPGSFA**HTLVDIAGEPRHAAQA**TGVSGDGKIVIGMKVPDDPFA
ITVGFQYIDGHLQPLEAVRPQCSVYPNGITPDGTVIVGTNYAIGMGSVAVKWVNGKVSELPMLPDTLD

SVASAVSADGRVIGGNRNINLGASVAVKWEDDVITQLPSLPDAMNACVNGISSDGSIIVGTMVDVSWR
NTAVQWIGDQLSVIGTLGGTTSVASAISTDGTVIVGGSENADSQTHAYAYKNGVMSDIGTLGGFYSLA
HAVSSDGSVIVGVSTNSEHRYHAFQYADGQMVDLGTLGGPESYAQGVSGDGKVIVGRAQVPSGDWHAF
LCPFQAPSPAPVHGGSTVVTSQNPRGMVDINATYSSLKNSQQQLQRLLIQHSAKVESVSSGAPSFTSV
KGAISKQSPAVQNDVQKGTFLSYRSQVHGNVQNQQLLTGAFMDWKLASAPKCGFKVALHYGSQDALVE
RAALPYTEQGLGSSVLSGFGGQVQGRYDFNLGETVVLQPFMGIQVLHLSREGYSEKNVRFPVSYDSVA
YSAATSFMGAHVFASLSPKMSTAATLGVERDLNSHIDEFKGSVSAMGNFVLENSTVSVLRPFASLAMY
YDVRQQQLVTLSVVMNQQPLTGTLSLVSQSSYNLSF

Processing sites that assiste in the secretion of the polypeptide from the cytoplasm and its release into the periplasm are located after aminoacid 31 (based on PSORT prediction and/or after aminoacid 47 similar to experimentally determined processing sites in other bacterial autotransporter molecules (e.g. BrkA from B.pertussis). Hence, the mature form of the Cpn0796 product is as follows:

SEQ ID NO: 81

HTLVDIAGEPRHAAQATGVSGDGKIVIGMKVPDDPFAITVGFQYIDGHLQPLEAVRPQCSVYPNGITP
D
GTVIVGTNYAIGMGSVAVKWVNGKVSELPMLPDTLDSVASAVSADGRVIGGNRNINLGASVAVKWEDD
VITQLPSLPDAMNACVNGISSDGSIIVGTMVDVSWRNTAVQWIGDQLSVIGTLGGTTSVASAISTDGT
VIVGGSENADSQTHAYAYKNGVMSDIGTLGGFYSLAHAVSSDGSVIVGVSTNSEHRYHAFQYADGQMV
DLGTLGGPESYAQGVSGDGKVIVGRAQVPSGDWHAFLCPFQAPSPAPVHGGSTVVTSQNPRGMVDINA
TYSSLKNSQQQLQRLLIQHSAKVESVSSGAPSFTSVKGAISKQSPAVQNDVQKGTFLSYRSQVHGNVQ
NQQLLTGAFMDWKLASAPKCGFKVALHYGSQDALVERAALPYTEQGLGSSVLSGFGGQVQGRYDFNLG
ETVVLQPFMGIQVLHLSREGYSEKNVRFPVSYDSVAYSAATSFMGAHVFASLSPKMSTAATLGVERDL
NSHIDEFKGSVSAMGNFVLENSTVSVLRPFASLAMYYDVRQQQLVTLSVVMNQQPLTGTLSLVSQSSY
NLSF

Or

SEQ ID NO: 82

TGVSGDGKIVIGMKVPDDPFAITVGFQYIDGHLQPLEAVRPQCSVYPNGITPDGTVIVGTNYAIG
MGSVAVKWVNGKVSELPMLPDTLDSVASAVSADGRVIGGNRNINLGASVAVKWEDDVITQLPSLP
DAMNACVNGISSDGSIIVGTMVDVSWRNTAVQWIGDQLSVIGTLGGTTSVASAISTDGTVIVGGS
ENADSQTHAYAYKNGVMSDIGTLGGFYSLAHAVSSDGSVIVGVSTNSEHRYHAFQYADGQMVDLG
TLGGPESYAQGVSGDGKVIVGRAQVPSGDWHAFLCPFQAPSPAPVHGGSTVVTSQNPRGMVDINA
TYSSLKNSQQQLQRLLIQHSAKVESVSSGAPSFTSVKGAISKQSPAVQNDVQKGTFLSYRSQVHG
NVQNQQLLTGAFMDWKLASAPKCGFKVALHYGSQDALVERAALPYTEQGLGSSVLSGFGGQVQGR
YDFNLGETVVLQPFMGIQVLHLSREGYSEKNVRFPVSYDSVAYSAATSFMGAHVFASLSPKMSTA
ATLGVERDLNSHIDEFKGSVSAMGNFVLENSTVSVLRPFASLAMYYDVRQQQLVTLSVVMNQQPL
TGTLSLVSQSSYNLSF

In silico analysis of the protein encoded by Cpn0796 also demonstrates a C-terminal domain comprising approximately residues from 1 to 648. FIG. 8 illustrates Cpn0796. As shown in FIG. 8, Cpn0796 forms a beta-barrel structure and is capable of forming a pore across the bacterial outer membrane (OM). As is typical of 'autotransporter' molecules, after being secreted across the bacterial inner membrane into the periplasm through an N-terminal signal peptide mechanism, the molecule may form a pore in the OM through which the N-terminal domain may pass (the 'passenger' domain) to the outside of the bacterial cell. Also, these molecules may either remain anchored to the bacterial surface or undergo a proteolytic cut which releases the 'passenger domain'

or a portion of it into the medium surrounding the bacterial cell an example of which is represented in the following sequence:

SEQ ID NO: 83

MKFMKVLTPWIYRKDLWVTAFLLTAIPGSFA**HTLVDIAGEPRHAAQ**ATGV
SGDGKIVIGMKVPDDPFAITVGFQYIDGHLQPLEAVRPQCSVYPNGITPD
GTVIVGTNYAIGMGSVAVKWVNGKVSELPMLPDTLDSVASAVSADGRVIG
GNRNINLGASVAVKWEDDVITQLPSLPDAMNACVNGISSDGSIIVGTMVD
VSWRNTAVQWIGDQLSVIGTLGGTTSVASAISTDGTVIVGGSENADSQTH
AYAYKNGVMSDIGTLGGFYSLAHAVSSDGSVIVGVSTNSEHRYHAFQYAD
GQMVDLGTLGGPESYAQGVSGDGKVIVGRAQVPSGDWHAFLCPFQAPSPA
PVHGGSTVVTSQNPRGMVDINATYSSLKNSQQQLQ

RLLIQHSAKVESVSSGAPSFTSVKGAISKQSPAVQNDVQKGTFLSYRSQVHGNVQNQQLLTGAFM
DWKLASAPKCGFKVALHYGSQDALVERAALPYTEQGLGSSVLSGFGGQVQ
GRYDFNLGETVVLQPFMGIQVLHLSREGYSEKNVRFPVSYDSVAYSAATS
FMGAHVFASLSPKMSTAATLGVERDLNSHIDEFKGSVSAMGNFVLENSTV
SVLRPFASLAMYYDVRQQQLVTLSVVMNQQPLTGTLSLVSQSSYNLSF

Also shown in FIG. 8, amino acid residues 365-385 represent an alpha helix conformation that spans the beta barrel pore

The N-terminal passenger domain may be cleaved via a specific proteolytic action from the membrane-anchored pore structure. A linker domain comprising the peptide sequence PSPAPV (**SEQ ID NO: 84**) as shown in bold in the following sequence illustrates a site at which cleavage of the N-terminal passenger domain may occur:

SEQ ID NO: 85

HTLVDIAGEPRHAAQATGVSGDGKIVIGMKVPDDPFAITVGFQYIDGHLQPLEAVRPQCSVYPNG
ITPDGTVIVGTNYAIGMGSVAVKWVNGKVSELPMLPDTLDSVASAVSADGRVIGGNRNINLGASV
AVKWEDDVITQLPSLPDAMNACVNGISSDGSIIVGTMVDVSWRNTAVQWIGDQLSVIGTLGGTTS
VASAISTDGTVIVGGSENADSQTHAYAYKNGVMSDIGTLGGFYSLAHAVSSDGSVIVGVSTNSEH
RYHAFQYADGQMVDLGTLGGPESYAQGVSGDGKVIVGRAQVPSGDWHAFLCPFQA**PSPAPV**HGGS
TVVTSQNPRGMVDINATYSSLKNSQQQLQRLLIQHSAKVESVSSGAPSFTSVKGAISKQSPAVQN
DVQKGTFLSYRSQVHGNVQNQQLLTGAFMDWKLASAPKCGFKVALHYGSQDALVERAALPYTEQG
LGSSVLSGFGGQVQGRYDFNLGETVVLQPFMGIQVLHLSREGYSEKNVRFPVSYDSVAYSAATSF
MGAHVFASLSPKMSTAATLGVERDLNSHIDEFKGSVSAMGNFVLENSTVSVLRPFASLAMYYDVR
QQQLVTLSVVMNQQPLTGTLSLVSQSSYNLSF

The N-terminal peptide may be secreted to be exposed on the bacterial cell surface and can also become detached via the proteolytic event described above. The peptide may form a structural conformation known as beta-propellers indicated in the following sequence:

SEQ ID NO: 86

HTLVDIAGEPRHAAQATGVSGDGKIVIGMKVPDDPFAITVGFQYIDGHLQPLEAVRPQCSVYPNG
ITPDGTVIVGTNYAIGMGSVAVKWVNGKVSELPMLPDTLDSVASAVSADGRVIGGNRNINLGASV
AVKWEDDVITQLPSLPDAMNACVNGISSDGSIIVGTMVDVSWRNTAVQWIGDQLSVIGTLGGTTS
VASAISTDGTVIVGGSENADSQTHAYAYKNGVMSDIGTLGGFYSLAHAVSSDGSVIVGVSTNSEH
RYHAFQYADGQMVDLGTLGGPESYAQGVSGDGKVIVGRAQVPSGDWHAFLC

Furthermore, the N-terminal passenger domain can also possess a specific protease activity, such as a serine protease-like activity. In addition to acting on a variety of substrates, the protease activity may act on the membrane anchored form of the molecule such that the N-terminal passenger domain is cleaved off form the surface of the chlamydial cell. The serine protease like activity is supported by the presence of a consensus serine protease triad of adequately spaced amino acid residues (namely H, D and S) which can be located on the virtual structure of the 'passenger' domain modelled on a set of experimentally-determined templates, e.g. 1nr0 (PDB identification code)

Based on the above analysis, the gene Cpn0796 gene encodes for a protein which promotes its own secretion on the EB surface and may also mediate or promote its own release into the surrounding medium. The secreted passenger peptide has several activities, including:

1. actin binding peptide, part of a chlamydial surface layer, and instrumental to the process of establishing the host cell infection
2. specific protease activity within the host cell cytoplasm instrumental to the intracellular survival of infecting chlamydiae.
3. specific activity within the host cell cytoplasm to down regulate expression of selected genes, either by repressing their transcription and/or by repressing their translation (m-RNA degradation)
4. cooperation with the products of genes Cpn0794, 0795, 0797, 0798, 0799
5. another function of the above N-terminal beta propeller domain is the regulation/ modulation of the activity of a cytosolic protease of the host cell in order to alter host cell properties in favour of chlamydial development, survival or persistence. See Fulop V, Bocskei Z, Polgar L. in "Prolyl oligopeptidase: an unusual beta-propeller domain regulates proteolysis." Cell. 1998 Jul 24;94(2):161-70.

The proteins encoded by Cpn0794, Cpn0797, Cpn0798, Cpn0799 - all comprising variants of the above described Cpn0796 structure - also provide beta propeller structures with activities similar and/or complementary to the ones described above.

Thus, a family of proteins cooperating to a common function either by generating - through events of site specific recombination - new molecules with structures and activities similar to the above described Cpn0796 product, OR by independently contributing to a multi-protein structure requiring a coordinated action of several related components.

FIG. 9 illustrates an alignment of the C-terminal domains of the proteins encoded by C.pneumoniae genes Cpn0795 and Cpn0796. As seen in FIG. 9, beta barrel domains of Cpn0795 or Cpn0796 include MKDLGTLGG (**SEQ ID NO: 87**), SXDGK (**SEQ ID NO: 88**) VIVG (**SEQ ID NO: 89**), VIXG (**SEQ ID NO: 90**) or HAF (**SEQ ID NO: 91**).

Fourth Group of Proteins

Cpn0498

So in this case the triple parallel-screening evaluation, with two positive and one negative result, brought to the identification of a previously unknown antigen (ie an

antigen with unknown biological function) having, according to current views, just the desirable basic properties in terms of antigenic function of a vaccine candidate. Further characterization of Cpn antigen data is included in *Finco et al.*, "Identification of New Potential Vaccine Candidates Against Chlamydia pneumoniae by Multiple Screenings," Vaccine, 23 (2005) 1178-1188, incorporated herein in its entirety.

Example 5

Background

The main stages in the *Chlamydial* life cycle are:

(i) the binding to the host cell surface and entry into the cytoplasm through a specialised vacuole (the *Chlamydial* inclusion) by an extracellular spore-like infective form, called the elementary body (EB); and

(ii) the conversion of the EB to a non-infective replicative form called a reticulate body (RB) that replicates by binary fission a number of times within the inclusion to form a microcolony.

The sets of genes which are expressed in the various stages of the *Chlamydial* life cycle and the signals that trigger the passage from one stage to the next are largely unknown and still need investigation.

Protein microarrays are used for high throughput protein analysis by detecting proteins and monitoring their expression levels. Through use of protein microarrays, complex screening of thousands of proteins and interactions with proteins may be performed in parallel. A protein array typically includes a surface, such as glass, membrane, microtiter wells, mass spectrometer plates, beads or other particles, for binding ligands, proteins, or antibodies. For example, antibodies may be bound to the microarray to form a capture array. The capture array may be contacted with a biological sample to quantify the proteins in the biological sample. Also, proteins may be bound to the microarray and contacted with a biological sample to quantify protein-protein or protein-ligand interactions. Thus, protein microarrays may also be used in diagnostics in which multiple immunoassays may be conducted in parallel such that levels of proteins in different samples may be quantified and compared for applications in the treatment or diagnosis of disease.

For example, in a capture array, antibodies are bound to the microarray and exposed to a biological sample. Proteins and ligands that bind to the antibody array may be detected by direct labelling of the bound proteins. If a higher sensitivity or specificity is desired, a sandwich technique may be employed in which pairs of antibodies are directed to the same protein ligand. This technique is particularly useful if the amount of protein to be detected is low or if there are modifications to the protein. In addition, the use of sandwich assays minimizes the risk of cross-reactivity in highly multiplexed assays by providing dual level target recognition, i.e. two levels of specificity for each locus in the array. Alternatively, the bound proteins may be detected via label-free detection methods such as including mass spectrometry, surface plasmon resonance and atomic force microscopy. This technique is useful if modification or alteration of the protein is to be avoided.

Also, Large-scale functional chips containing large numbers of immobilized purified proteins may be used to assay a wide range of biochemical functions, such as protein interactions with other proteins, drug-target interactions, enzyme-substrates, etc. Such proteins may be purified from an expression library, for example, and the protein

array can be used to screen libraries to select specific binding partners, including antibodies, synthetic scaffolds, peptides and aptamers. In this way, 'library against library' screening can be carried out, such as screening of drug candidates in combinatorial chemical libraries against an array of protein targets identified from genome projects.

Protein microarray technology permits analysis of the proteins themselves rather than inferring protein function, interactions and characteristics through mRNA expression. In many cases, mRNA expression does not correlate accurately with protein abundance. Furthermore, mRNA expression analysis does not provide sufficient information on protein-protein interaction or post-translational modifications. Thus, direct analysis of proteins via protein microarrays provides an advantage by providing more accurate information of proteins and protein-protein interactions that may not be readily available through measurment of mRNA expression.

Current DNA microarray techniques permit profiling of gene expression at the mRNA level as a function of the cellular state. This can lead to the identification of genes or clusters of genes whose up- or down-regulation is associated to a particular state of the cell and to the identification of therapeutically relevant targets. Using this technology, DNA fragments representing specific portions of all genes belonging to a given organism (the fragments can be derived from cDNA libraries or can be obtained by PCR amplification and chemical synthesis) are chemically bound to the surfaces of solid supports (chips) at high densities and in an ordered manner. Currently up to 10, 000 DNA fragments or 250, 000 oligonucleotides can be spotted onto a single squared centimetre of chip surface. The DNA chips are then utilised to define which of the spotted genes are transcriptionally active in a particular cellular population. To do so, RNA is prepared, labelled with fluorescent dyes and finally hybridised to the DNA fragments fixed to the surface of the chip. By using an appropriate computer-assisted fluorescence detector, the fluorescence signals emitted by each spot upon excitation with a laser beam is carefully quantified to define the transcription activity of all the arrayed genes.

CPn DNA microarrays have been developed to look at the transcriptional events which occur when a given CPn pathogen gets into contact with the host cells, both in in vivo and in vitro settings. DNA chips carrying the entire genome of a particular bacterium, such as the CPn bacterium can be prepared in a very short period of time so that whole genome expression analysis can be determined.

Experimental Methodology

Specifically, a genomic DNA (open reading frame probes) microarray approach for gene expression in CPn bacteria was adopted. In this respect, an array was prepared for the analysis of the CPn life cycle on the basis of the published annotation of the complete genome. The Chlamydia DNA chips carry about 1000 PCR-derived DNA fragments, which have an average size of 400-700bp and correspond to internal portions of all CPn annotated genes.

Results 5

Table 3(i)-(xi) shows transcriptional activity for expressed genes for CPn EB selected by microarray. The data in Tables 3(i)-(iv) shows different mRNAs in order of abundance from cells in their infectious "spore-like" (EB) form. Data in Tables 3(v)-

(xi) correlates and summarizes mRNA expression levels of genes for CPn. The cells were used at the end of their cycle where gene expression is likely to be at its highest. As values less than approximately 10000 is likely to be background, the top set of proteins (approx top 30) with more intense signals are likely to be the most interesting proteins.
A review of the values for the expressed genes indicates that three of the FACS positive CPn antigens (CPn0331 (hypothetical), CPn0234 (hypothetical) and CPn0572 (hypothetical) are all highly expressed genes.

Table 3(v)-(xi) shows the transcriptional activity for expressed genes for CPn EB selected by microarray. The Table shows different mRNA in order of abundance from cells in their infectious "spore-like" (EB) form. The cells were used at the end of the cycle where gene expression is likely to be at its highest. A review of Table 3(i)-(iv) and (v)-(xi) indicates that CPn antigens CPn0558 (OmcA), CPn0331 (hypothetical), CPn0539 (Pmp19), CPn0234 (Hypothetical) and CPn0572 (Hypothetical) are all relatively highly expressed genes.

Where possible, attempts were made to place the transcriptional activities disclosed in Table 3(v)-(xi) in the context of the Chlamydia developmental cycle In this respect, Chlamdydia late gene products have been described more frequently than early gene products. This is primarily because of the presence of late gene products in EBs but not RBs and that it is easier to study EBs rather than RBs.

In addition, late gene functions appear to be predominantly those associated with the terminal differentiation of RBs back to EBs (Shaw et al., Mol Microbiology 37(4), 2000, 913-925). Late gene products appear to function in the termination of bacterial cell division and constitute structural components and remodelling activities involved in the formation of the cross-linked outer membrane complex that functions in the attachment and invasion of new host cells. By way of example, an important aspect of the secondary differentiation process (RB to infectious EB) is the expression of genes that encode proteins that form the highly disulfide cross-linked bacterial outer membrane (OM) complex. It is thought that several late cycle genes encode proteins with potential roles in the formation and maturation of the OM complex, a crucial step in the development of infectious EBs (see Belland et al., PNAS (USA) 100(14), 2003, 8478-83). The late genes omcA and omcB encode two cysteine-rich OM proteins that interact with the major OM protein (OmpA) to form this complex. A key protein component of the OM complex, the OmcB protein, has been found to undergo post-translational proteolytic processing. We have found that OmcB and OmcA show high levels of transcriptional activity (see top of Table 3(ii)). Cpn 0384 whose CT equivalent is CT046 (hctB) has been shown to be associated with differentiation from RB to EB (see Belland et al., PNAS (USA) 100(14), 2003, 8478-83). We also found Cpn0384 to have relatively high levels of transcriptional activity (again see top of Table 3(v)-(xi)). Other Cpn antigens thought to be involved in the Type III secretion system were found to have moderate expression levels in terms of transcriptional activity. This finding appears to be in line with published commentary where it is thought that while transcription of the two putative structural components of the Type III secretion system (yscJ and yscN (Cpn669)) begins at mid-cycle, export of effector molecules may be at a different stage of the developmental cycle.

Table 3(v)-(xi) indicates that high transcriptional activity was observed for Cpn0539 (CT412) which corresponds with a 98Kda protein known either as PmpA or Pmp19. Even though the Pmp19 protein demonstrates relatively "high" levels of transcriptional activity, this result is interesting because mRNA abundance for pmp19 does not seem to correlate with protein abundance. In this respect, results from our laboratory have shown that (i) Pmp19 was not detected in either 2D maps, Western Blots or FACS analysis studies which suggests that the pmp19 protein either is not surface exposed even though high levels of mRNA are expressed or that (ii) Pmp19 protein is expressed but processed or degraded by proteolytic digestion rendering it undectable by immunoblot analysis. The results in our laboratory are confirmed by others. In this respect, Grimwood et al (2001) Infection and Immunity 69(4) 2383-2389 have shown that transcriptional profiles were detected for each of the Chlamydia pneumoniae 21 Pmp genes demonstrating that all pmp genes are transcribed during infection. Since each of the Pmp genes was transcribed, Grimwood et al (2001) evaluated protein expression by immunoblotting of Chlamydia pneumoniae CWL029 EB lysates using peptide-specific antisera. Interestingly, no Pmp-specific reactivity was detected for sera from either PmpA (Pmp19) or PmpB/C and PmpD gene by immunoblot analysis regardless of high antipeptide reactivity. This result suggested that these proteins either are not stable or not translated. These findings demonstrate that there appears to be a variation in Pmp expression for the *Chlamydia pneumoniae* family of 21 polymorphic membrane proteins (Pmps) which are predicted to be localised to the bacterial outer membrane. The function of Chlamydial Pmps remains unknown, although based on sequence prediction and experimental testing, these Pmps are regarded as surface proteins and thus, likely to be critical for Chlamydial virulence. Like the Inclusion (Inc) Membrane proteins, the Pmp proteins are regarded, at present, as unique to the Chlamydiae family (see Rockey et al (2000) Infection and Immunity 69(10) 5473-5479). The findings disclosed here and by others, such as Grimwood et al, demonstrates that the Chlamydia organism appears to expend a considerable metabolic cost in Pmp transcription, such as Pmp19 transcription, despite the potential lack of production of a functional Pmp proteins, such as the Pmp19 protein.

Materials and Methods (Examples 6-8) (Reference Section II)

T cell Epitope prediction and peptide synthesis

T cell epitope prediction was carried out on the genomic sequence of *C. pneumoniae* CWL029 strain (Accession numbers NC_000922 or AE001363) using the BIMAS algorithm [24]. Synthetic peptides (purity > 80%) were synthesized by Primm Srl (Milan, Italy), suspended in 100% DMSO and kept at -20° C before use.

RMA-S/A2 cell line and HLA-A2 transgenic and non transgenic mice

The T cell lymphoma murine cell line RMA-S stably transfected with HLA-A2 (RMA-S/A2, H-2^b , TAP2$^-$), was kindly provided by Dr. Barnaba, Università degli Studi "La Sapienza", Rome, Italy, and cultured at 37° C in RPMI-1640 (GIBCO) supplemented with heat inactivated 10% FCS, 100 IU/ml penicillin/streptomycin, 2 mM Lglutamine (GIBCO) and 5×10–5 M 2-ME (Sigma). H2-b HLA-A2 transgenic mice [35] were housed in a pathogen-free environment and screened for HLA-A2 expression by FCM carried out on total blood samples using the BB7.2 anti-A2 mAb [48]. Only mice with percentages of A2 expressing cells higher than 70-80 % were

used for DNA immunization and *C. pneumoniae* infection experiments. Animals which showed no HLA-A2 expression were mated in order to obtain an HLA-A2 non transgenic population, to be used as a control in the experiments.

Epitope stabilization assay

RMA-S/A2 cells (3-5 x 10^5/well) were seeded in serum-free RPMI medium, supplemented with human β2 microglobulin (3 μg/ml, Sigma), without or with the test peptide (10μM). Following overnight incubation at 26°C in humidified 5% CO_2 atmosphere, cells were shifted to 37° C for 2 h before determining the HLA-A2 expression level at the cell surface using the BB7.2 anti-A2 mAb and a PE-conjugated anti-mouse IgG (Jackson ImmunoResearch). Fluorescence intensity on living cells, which did not incorporate propidium iodide, was analyzed by FCM. As controls, corresponding samples without peptide and samples with peptide but treated only with the anti-mouse secondary antibody, were used.

Infection and DNA immunization of HLA-A2 transgenic and non transgenic mice

Transgenic mice were intranasally infected twice with a month interval, using 5x10^5 *C. pneumoniae* FB/96 EBs [4] diluted in 50 μl of PBS. *C. pneumoniae* antigen coding genes were amplified by PCR using FB/96 genomic DNA, cloned into plasmid pcmvKaSF2120 [49] and verified by DNA sequence analysis. Fifty μg of endotoxin free recombinant plasmid DNA, diluted in Dulbecco's phosphate buffer (GIBCO), were injected into the tibialis muscle of mice at days 0, 21 and 35.

$CD8^+$ T cells isolation and IFN-γ determination by ELISpot assay

Splenocytes from DNA immunized mice were prepared one week after the third immunization using Cell Strainer (Falcon) filters. Following red blood cells lysis, $CD8^+$ T cells from spleen cells suspensions were enriched by positive selection using magnetic activated cell sorting (MACS-Miltenyi Biotec) with CD8a (Ly-2) microbeads. $CD8^+$ T cells purity was higher than 90%, as determined by FMC. Multiscreen 96-well nitrocellulose plates (Millipore) were coated with 5 μg/ml of the anti-mouse IFN-γ antibody (R4–6A2, PharMingen) in 100 μl of carbonate buffer, pH 9.2. After overnight incubation at 4°C, plates were saturated at 37°C with 200 μl of BSA (1%) in PBS for 2 h. Purified $CD8^+$ (5x10^4) were added in a total volume of 200 μl/well in the presence of irradiated (3,000 rad) spleen cells from non immunized HLA-A2 transgenic mice as a source of antigen-presenting cells (2x10^5/well), 10 μg/ml of peptide and 10U/ml of human r-IL-2 (Chiron Corporation). After incubation for 20 h at 37° C, 5% CO_2, plates were washed and developed for bound IFN-γ using sequential incubations with biotinylated antimouse IFN-γ (XMB1.2, PharMingen), ExtrAvidin-alkaline phosphatase and substrate BCIP/NBT (Sigma) dissolved in water. Spots were enumerated in each well using a dissecting microscope. Medium containing an irrelevant peptide or without peptide was used as negative controls, while positive controls were represented by $CD8^+$ T cells treated with ConA (5 μg/ml).

In vitro* cultures and flow cytometric analysis of splenocytes from transgenic and non transgenic mice infected with *C. pneumoniae

Splenocytes from infected mice were isolated one week after the second infection with *C. pneumoniae* Ebs. For *ex vivo* analysis of IFN-γ production, 2x10^6 splenocytes

were seeded in the presence of the test peptide (10μg/ml) and anti-mouse CD28 antibody (1μg/ml, PharMingen) as co-stimulus. After a two h incubation at 37° C, 5 % CO_2, Brefeldin A (10 μg/ml, Sigma) was added and the incubation was extended for 4 additional hours. Following two washes with PBS, cells were permeabilized, fixed and stained both with anti-murine-IFN-γ-(PE), anti-murine CD8 (APC) and anti-murine-CD69 (FITC) and the corresponding isotypes. Positive controls for cytokine production were performed on cells stimulated with anti-mouse CD3 and CD28 antibodies (1 μg/ml, PharMingen) . Cells cultured either in the absence of peptide or pulsed with the HepB negative control peptide were used as negative controls. All samples were analyzed using a FACS LSRII flow cytometer (Becton Dickinson). For analysis of IFN-γ production by short term T cell lines, 5-10x10^6 splenocytes from infected mice were cultured for 6 days in the presence of the test peptide (20 μg/ml), with rIL-2 (10 μg/ml) being added after the first two days. At the end of the incubation period, cells were washed twice in RPMI, pulsed again for 6 h in the presence of the test peptide (10μg/ml), 1x10^5 freshly prepared CD8 depleted antigen presenting cells from HLA-A2 transgenic mice (irradiated at 3000 rad) and anti-mouse CD28 antibody (1μg/ml, PharMingen) as co-stimulus. After a two h incubation at 37° C, 5 % CO_2, Brefeldin A (10 μg/ml, Sigma) was added, the incubation was extended for 4 additional hours and IFN-γ production was analyzed by FCM.

Example 6

In silico analysis of *Chlamydia pneumoniae* genome and prediction of HLA-A2 T cell epitopes

The genome of the *Chlamydia pneumoniae* CWL029 strain was used to predict 9mer peptide sequences with high probability to bind class I HLA-A2 molecules. The analysis was carried out using the predictive algorithm available at the NIH Bioinformatics & Molecular Analysis Section Web server (http://bimas.cit.nih.gov/), which ranks potential MHC binders according to the predictive half-time dissociation of peptide/MHC complexes [24]. Since some *Chlamydia*l proteins have been reported to induce autoimmune responses [25-28], we restricted our search to a subset of proteins, distinctive of the *Chlamydia* genus, consisting of 13 protein identified as members of the type III secretion system, 17 Polymorphic Membrane Proteins (PMP) and 19 additional proteins, 5 of which already identified as EB surface antigens [4]. The predicted binding score of 157.22, obtained for the well characterized HIV-1 p17 gag epitope ^{77}SLYNTVATL^{85} [29], was taken as an arbitrary cut-off for peptide selection. A total of 55 potential *C. pneumoniae*-derived T cell epitopes, belonging to 31 different proteins, were identified (Table I), which had predicted binding scores ranging from 156.77 to 42,485.263

***In vitro* binding of peptides to HLA-A2**

The capacity of the selected peptides to bind to HLA-A2 was assessed using an *in vitro* MHC class I stabilization assay, carried out with the murine transporter associated with antigen processing (TAP)-deficient cell line RMA-S/A2, stably transfected with the human class I A2 gene. MHC class I molecules, cultured at 37° C, are unstably expressed on the cell surface of TAP-deficient cells [30-32]. Culturing the cells at 37° C in the presence of binding peptides, results in formation of a more stable MHC/peptide complex which can be monitored by flow cytometric analysis. RMA-S/A2 cells were therefore cultured overnight at 26° C in the presence of the

test peptides, shifted to 37° C for 2 hours and the surface level of stabilized A2 molecules was quantified by direct staining with an anti-HLA-A2 specific mAb. Two known HLA-A2 restricted CTL epitopes were used as positive controls for binding to A2, the HIV-1 p17 gag peptide [29] and the influenza matrix M1 protein peptide FluMP58 [33], while the Hepatitis B virus envelope antigen peptide HbenvAg125 (HepB) was used as a negative control [34].

Results 6

The binding results obtained are shown in Table 4 and allowed the identification of 15 peptides with a net mean fluorescence intensity (Net MFI) higher than 92.3, corresponding to the value obtained with the HIV-1 p17 gag positive control peptide, 8 peptides with a Net MFI intermediate between the values 92.3 and 63.1, obtained with the two positive control peptides, and 12 peptides with an Net MFI ranging between 29.6 and 63. Fifteen of the in silico predicted peptides (27.2 %) did not confer stabilization to the A2 molecules, showing a Net MFI lower than 14, obtained with the HepB negative control peptide.

Example 7

Some HLA-A2 binders are recognized by $CD8^+$ T cells from DNA-immunized transgenic mice

The *in vitro* assay with RMA-S/A2 cells allowed the definition of a set of peptides which were able to bind and stabilize the HLA-A2 molecules on the cell surface. To gain information about the possibility that the predicted epitopes could indeed be generated by *in vivo* processing of the antigens from which they were derived, peptide recognition by $CD8^+$ T cells was studied under conditions in which the related complete antigen was intracellularly expressed and presented *in vivo*. The full-length ORF sequences coding for 13 *Chlamydia*l proteins, including a total of 24 predicted peptides, were cloned into a suitable DNA expression vector and each recombinant plasmids was used to immunize distinct groups of transgenic mice expressing a chimeric class I molecule composed of the α1 and α2 domains of HLA-0201 and the α3 domains, transmembrane and cytoplasmic, of H-$2K^b$ [35].

The ORF sequences were selected among those containing either one or more epitopes positive in the *in vitro* assay or a combination of positive and negative epitopes. The ORF sequence corresponding to the outer membrane protein A (OMPA, CPn 0695) was included in this analysis, since human MHC-I-restricted epitopes have already been reported for this protein in *C. trachomatis* [18;36]. One coding sequence, related to gene CPn 0131 was chosen, which included four epitopes, all negative in the *in vitro* stabilization assay. After three immunization cycles, transgenic mice were sacrificed, spleen $CD8^+$ T cells were isolated, stimulated for 20 hour with the corresponding peptide and *ex vivo* IFN-γ production was assessed using an enzyme-linked immunospot (ELISpot) assay.

Results 7

DNA-mediated expression of the ORFs including peptides CH-6 (CPn 0811), CH-7 (CPn 0623), CH-10 (CPn 0828), CH-13 (CPn 0695, OMPA) and CH-37 (CPn 0210) were associated with numbers of spot forming cells (SFC) significantly higher than those obtained with the HepB unrelated peptide, whereas some peptides related to antigens coded by genes CPn 0131, CPn 0323 and CPn 0062 induced SFC values only 2-3 times higher than the HepB control peptide (Table 5). Peptides related to

antigens coded by genes CPn 0132, CPn 0322, CPn 0325, CPn 0415 and CPn 0728 did not induce any IFN-γ production (data not shown).

Example 8

To test the capacity of peptides to amplify specific $CD8^+$ T cell populations *in vitro*, some of these plasmids were used to repeat the DNA immunization experiment and to determine by flow cytometry the intracellular IFN-γ production by $CD8^+$ T cells, both *ex vivo* and after a 6 day stimulation in the presence of the relevant peptides. In the attempt to establish a direct correlation between IFN-γ production by $CD8^+$ T cells and HLA-A2 specific restriction, the experiment was carried out with both transgenic and non transgenic syngenic mice. The plasmids used contained genes CPn 0695, CPn 0811 and CPn 0823, including peptides CH-13, CH-6 and CH-7 respectively, which were highly positive in the *in vitro* binding and in the ELISpot assays and gene CPn 0323, including six different peptides, all of them with ELISpot values slightly higher than background

Results 8

The results of the experiment are summarized in Table 6, while representative dot plots from flow cytometric analysis of splenocytes stimulated with peptide CH-6 are shown in Fig. 4. When fresh spleen cells of DNA-immunized transgenic mice were pulsed with the tested peptides, only CH-6 or CH-7 induced relative fold increase (RFI) values about 5 times higher than those obtained pulsing the same cells with the HepB negative control peptide (Table 6, 4.58 and 5.2 RFI respectively).

When short term T cell lines (TCLs) instead of fresh splenocytes were used, a larger panel of peptides were able to trigger a significantly higher IFN-γ production by $CD8^+$ T cells (Table 6). In fact, in addition to peptides CH-6 and CH-7, also peptides CH-13, CH-44, CH-45 and CH-46 were recognized by $CD8^+$ T cell populations significantly larger than those induced by pulsing the same cells with the HepB peptide (RFI > 5). Importantly, peptide-induced IFN-γ production, appeared to be largely HLA-A2-dependent, since when the same experiments were carried out with non transgenic mice, the RFI values obtained were reliably lower (Table 6). The fact that non transgenic and transgenic spleen cells were both efficiently activated using the polyclonal stimulus (anti-CD3/anti-CD28), reinforced the hypothesis that the lower $CD8^+$ T cells induction in non transgenic mice was due to the absence of specific interactions between the peptides and the human HLA-A2 molecules.

$CD8^+$ T cells of transgenic mice infected with *C. pneumoniae* recognize HLA-A2 binders *in vivo*

It has been recently shown that infection of mice with *C. pneumoniae* elicits a pathogen-specific murine class I-restricted immune response [22]. Therefore, we asked whether any of the A2 *in vitro* binders could be recognized by specific $CD8^+$ T cells that are clonally selected during the immune response raised against the corresponding native antigen in *C. pneumoniae* infected cells.

To address this issue, HLA-A2 transgenic mice were intranasally infected with a non lethal dose of *C. pneumoniae* EBs and challenged with an equal dose of bacteria one month later, before being sacrificed to obtain splenocytes that were used to measure IFN-γ production by $CD8^+$ T cells. Since no appreciable IFN-γ-production could be observed if splenocytes from infected mice were tested directly *ex vivo* (data not

shown), spleen cells were cultured with each individual peptide or with the HepB irrelevant peptide for 6 days. The resulting short-term TCLs were then pulsed again for 6 hours with the same peptides and intracellular IFN-γ production by $CD8^+$ T cells was assessed. The results obtained with 40 tested peptides are shown in Fig. 5A. Sixteen peptides (CH-2, CH-7, CH-8, CH-10, CH-13, CH-15, CH-20, CH-21, CH-28, CH-35, CH-37, CH-45, CH-46, CH-47, CH-50 and CH-55) elicited the strongest $CD8^+$ responses (1 to 7.1 % of IFN-γ-producing $CD8^+$ T cells), while 19 peptides elicited low but consistent responses (percentages of $CD8^+$/IFN-γ^+ T cells between 0.3 and 0.9). Five peptides did not induce percentages of IFN-γ-producing $CD8^+$ T cells significantly higher than those observed in response to the HepB control peptide.

When eight among the most reactive peptides were used again to pulse splenocytes of both transgenic and non transgenic mice infected with *C. pneumoniae*, seven of them were recognized by specific $CD8^+$/IFN-γ^+ T cell populations present only in the transgenic mice, while peptide CH-7 was recognized by $CD8^+$ T cells present in both mice groups (Fig. 5B).

General Discussion of Results in Examples 6-8

In this work we have described peptides derived from *C. pneumoniae* antigens identified as putative T cell epitopes recognized by the common human class I MHC A2 haplotype.

Understanding *C. pneumoniae*-specific $CD8^+$ T cell-mediated immune response and designing protective vaccines rely on the possibility of identifying bacterial antigens or epitopes recognized by $CD8^+$ T cells. Whereas the induction of a CTL-dependent immune response is predictable in response to pathogens which replicate in the cellular cytosol, providing antigens which can enter the cellular MHC-I presentation pathway, in the case of *Chlamydiae* it is not immediately obvious which antigens are made available to the proteasome and how they reach the cytosol, since these bacteria have a stringent intravacuolar localization inside the infected cell.

We have chosen an *in vivo* system based on HLA-A2 transgenic mice to test which of the predicted peptides could be recognized by specific $CD8^+$ T cells following either DNA immunization with individual antigen coding genes or infection with *C. pneumoniae*. Our choice of a murine model is supported by previously published data. Wizel *et al.* [22], recently reported the first evidence that $CD8^+$ T cells specific for *C. pneumoniae* antigens are induced in infected mice, and identified bacterial-derived murine MHC-I-restricted T cell epitopes able to trigger either lysis of *C. pneumoniae* infected cells or *in vitro* inhibition of the pathogen intracellular growth. These findings seem to confirm that some *C. pneumoniae* antigens can indeed reach the cytosol of infected cells and enter the MHC-I presentation pathway, i.e. during remodeling that occurs during *Chlamydia* replication or following autolysis of developing bacterial particles [22].

Furthermore, Kuon *et al.* [42] recently reported the identification of 11 *C. trachomatis*-derived HLA-B27-restricted peptides, capable of stimulating $CD8^+$ T cells obtained from patients with *Chlamydia*-induced reactive arthritis. Importantly, 8 of them overlapped those selected by analyzing splenocytes of HLA-B27 transgenic mice infected with *C. trachomatis*, indicating that antigen processing can be closely

reproduced using the murine animal model, although differences between murine and human antigen processing and T cell repertoires have been hypothesized [43].

The experiment which we have performed with *C. pneumoniae* infected A2 transgenic mice revealed that at least 16 peptides were recognized by IFN-γ-positive $CD8^+$ T cell populations, which were actually expanded as a consequence of bacterial infection, since we could not detect IFN-γ production pulsing spleen cells from non infected transgenic mice with the same peptides (data not shown). These results suggest that the corresponding *Chlamydia*l antigen may be able to enter the MHC-I presentation pathway. The finding that a number of these peptides can also be recognized by specific $CD8^+$ T cells when the corresponding protein is separately expressed by DNA immunization, strongly reinforces the hypothesis that the responses observed with the infected mice are indeed specific for the *in silico* predicted peptide epitopes and their corresponding antigens. Importantly, the comparisons of peptide-induced IFN-γ-positive $CD8^+$ T cells in A2 transgenic and non transgenic mice, either immunized with DNA or infected with *C. pneumoniae,* indicate that this recognition event is largely A2-specific.
Though, we cannot rule out the possibility that some of the tested peptides are also able to interact with the murine class-I MHC molecules, as suggested by the result obtained with CH-7 in infected non transgenic mice (Fig. 5) and by the RFI values obtained with CH-7, CH-8 and CH-13 in DNA-immunized non transgenic mice (Table 6).

Both with DNA immunization and bacterial infection, we were able to show that the OMPA-derived CH-13 peptide induces a specific $CD8^+$ T cell response in A2 transgenic mice. These results appear to validate the choice of this animal model, since our observation that OMPA can enter the MHC-I presentation pathway correlates with the previous identification of HLA-A2-restricted and of murine MHC-I-restricted epitopes in OMPA proteins of *C. trachomatis* [18] and of *C. pneumoniae* [23] respectively. With the exception of CH-13 and CH-17, all the other peptides recognized by $CD8^+$ T cells of infected mice belong to *C. pneumoniae* antigens for which neither human nor murine T cell epitopes have been identified [22;23]. Interestingly, a couple of positively reacting peptides (CH-50 and CH-55) belong to the group of polymorphic outer membrane proteins [44;45], while most of the others are part of the group of Type III secretion system-related proteins [45;46]. Peptides CH-7 and CH-8, both included in protein T of the *Yersinia* outer protein (Yop) system [47] and CH-10, included in protein J, which is part of the same translocation system, appear to be particularly reactive in the assay with the infected mice (Fig. 5A).

This is also true for other peptides included in antigens which are again related to the type III secretion system, such as CH-45, CH-46, and CH-47, all present in the low calcium response protein D. Intriguingly, CH-8, which is the most reactive peptide in the assay with the infected mice, does not seem to be recognized by a specific T cell population when the corresponding antigen is expressed by DNA immunization (Tables 5 and 6). This may be due to different factors, i.e. low *in vivo* expression level of the injected DNA or altered protein conformation.

On the other hand, we should also consider the possibility that, following infection of mice with *C. pneumoniae,* this peptide is recognized by a $CD8^+$ T cell population

which is instead specific for an epitope derived from an unidentified *C. pneumoniae* antigen having a closely related sequence. Contrarily to CH-8, stimulation of spleen cells from infected transgenic mice with peptide CH-6 did not allow the detection of IFN-γ^+/CD8$^+$ T cells (Fig. 5A), but the same peptide was clearly reactive in the DNA immunization experiments (Tables 5 and 6). This may suggest that Low Calcium Response Protein H is not available for the cellular proteasome, but we could also assume either that the amount of peptide available to the MHC-presenting machinery is not sufficient to induce a cell response which is detectable with our assay, or that the reacting CD8$^+$ T cell population does not expand over the detection limit of our assay.

On the whole, the results presented here allowed the identification of a number of antigens which may be available for proteasome-mediated processing in the course of *C. pneumoniae* infections, proposing them as possible targets for a HLA-A2-dependent cellular immune response. Further analysis will be required to verify if the specifically induced CD8$^+$ T cells are able to recognize and induce lysis of peptide pulsed or *C. pneumoniae* infected mammalian cells and, possibly, to correlate the identified T cell epitopes with CD8$^+$ T cell populations naturally induced in *C. pneumoniae* infected patients. Importantly, the results obtained with DNA-mediated expression of distinct antigens, can represent an initial step towards the definition of a significant set of *C. pneumoniae* HLA-A2-restricted epitopes, which could eventually be combined in DNA minigenes in the attempt to induce a CTL-dependent anti-*Chlamydia* protective immune response

Example 9

Immunizations with Combinations of the First Antigen Group

The five antigens of the first antigen group (OmpH-like protein, pmp10, pmp2, Enolase, OmpH-like, CPn0042 and CPn00795 were prepared as described in the Materials and Methods Section above for Examples 1-4. The antigens are expressed and purified. Compositions of antigen combinations are then prepared comprising five antigens per composition (and containing 15 μg of each antigen per composition). CD1 mice are divided into seven groups (5-6 mice per group for groups 1 through 4; 3 to 4 mice for groups 5, 6 and 7), and immunized as follows:

Group	Immunizing Composition	Route of Delivery
1	Mixture of 5 antigens (15 μg/each) + CFA	Intra-peritoneal
2	Mixture of 5 antigens (15 μg/each) +AlOH (200μg)	Intra-peritoneal
3	Mixture of 5 antigens (15 μg/each) + AlOH (200μg) + CpG (10μg)	Intra-peritoneal
4	Complete Freunds Adjuvant (CFA)	Intra-peritoneal
5	Mixture of 5 antigens (5 μg/each) + LTK63 (5μg)	Intranasal
6	AlOH (200μg) + CpG (10μg)	Intra-peritoneal
7	LTK63 (5μg)	Intranasal

Mice are immunized at two week intervals. Two weeks after the last immunization, all mice are challenged by intravaginal infection with *Chlamydia pneumoniae* serovars.

Experiment 9 was repeated with another group of CPn antigens. These were:

CPn0385 (PepA), CPn0324 (LcrE), CPn0503 (DnaK), CPn0525 (Hypothetical) and CPn0482 (ArtJ). These antigens are combined and administered with and without alum and CpG as described in Experiment 9.

Summary

Applicants have identified a number of CPn proteins with desirable immunological and/or biological properties. Specifically, at least twelve CPn proteins have been identified which are capable of inducing the production of antibodies, which can neutralise, in a dose-dependent manner, the infectivity of *C. pneumoniae* in *in vitro* cell cultures. The induction of neutralising antibodies is important because it prevents infectious EBs from invading human tissues. Furthermore, at least six of these CPn proteins were also capable of attenuating *Chlamydial* (*C. pneumoniae)* infection in a *in vivo* hamster model. In addition, some of these CPn proteins were also capable of inducing not only adequate T-cell responses but also high serum levels of neutralising antibodies.

Apart from very recent unpublished results on pmp21, this is the first time that antisera to recombinant pmps (pmp2 and pmp10) are reported to have neutralising properties. Interestingly, whilst antiserum against CPn0525 gave negative *in vitro* results (ie no neutralising activity), the CPn0525 protein gave 97 per cent protection from spleen infection in an *in vivo* hamster immunisation assay (see Table 2) (ie a positive *in vivo* result). Likewise, whilst antiserum against Cpn0498 gave negative *in vitro* results (ie no neutralising activity), the CPn0498 protein gave 94 per cent protection from spleen infection in an *in vivo* hamster immunisation assay (ie a positive *in vivo* result). Thus a positive signal obtained in the FACS assay does not guarantee a corresponding positive *in vitro* neutralization activity and conversely a negative neutralization activity does not mean that a positive *in vivo* result can be excluded.

Some of the results obtained by screening the panel of recombinant antigens with the *C.pneumoniae in vitro* neutralization assay are shown in Table 2. Just by a cursory look at the 'current annotation' column it can be seen that both in Table 1 and 2 are listed antigens, like the members of the family of heterogeneous polymorphic membrane proteins (PMP), which, on the basis of published literature data, could be reasonably expected to be surface-exposed and possibly induce neutralizing antibodies, but there are also proteins which could be considered so far only hypothetical, and proteins which just on the basis of their current functional annotation could not be at all expected to be found on the bacterial surface.

The characterisation for the first time of some of these CPn proteins in terms of not only neutralising properties but also different score profiles in a panel of screening tests is an important contribution to the art because it facilitates the selective combination of CPn antigens with particular immunological and biological properties.

In conclusion, this paper describes a group of recombinant antigens which can induce antibodies inhibiting the infectivity of *C. pneumoniae in vitro* and have protective effects *in vivo*.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the

scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be covered by the present invention.

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[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CPn0449	0010-GST	Pmp 10, family of polymorphic outer membrane proteins	1-25	94503	5.22	40.24	Fig2	yes	267	92	0.0340*
CPn0013	6270-GST	Pmp 2,family of polymorphic outer membrane proteins	1-19	87459	5.84	34.99	Fig2	yes	87	92	0.0338*
CPn0482	6767-GST	ArtJ, Arginine binding protein?	?(*)	29070	5.45	20.16	Fig2	yes	105	43	0.2918
CPn0800	7111-GST	Eno, Enolase	no	46104	4.66	11.64	Fig2	yes	413	89	0.0005*
CPn0979	7306-GST	HtrA, chaperonin, serine protease	1-20	50142	6.39	28.67	Fig2	yes	249	0	0.9833
CPn301	6577-GST	OmpH-like outer membrane protein	1-21	17264	4.75	10.05	Fig2	yes	847	84	0.0067*
CPn0042	6296-GST	Cpn specific hypothetical, several possible paralogs, including the FACS+ Cpn0126	no	31.1	5.40	22.77	ND	no	80	84	0.0002*
CPn0795	7106-GST	Cpn specific hypothetical	no	39.5	7.50	14.84	ND	no	487	89	0.0109*
CPn0584	6878-GST	AtoS; Two-component sensor histidine kinase, AtoS-related	no	40.4	7.50	14.61	ND	no	270	74	0.3457
CPn0558	6850-GST	OmcA - predicted 9 kD cysteine rich, outer membrane protein, lipoprotein?	lipoprotein ?	7.4	7.56	20.45	ND	no	197	64	0.2061

TABLE 1

TABLE 3(C)

Expressed genes of Chlamydia pneumoniae EB selected by microarray

CPN gene	Annotation	CMedia
DN1+2		56951.62
CPn0332	CHLTR T2 Protein	52466.50
omcB	omcB 60 kDa Cysteine-Rich OMP	43304.22
omcA	omcA 9 kDa Cysteine-Rich Lipoprotein	42530.16
ompA	ompA Major Outer Membrane Protein	40478.10
CPn0678	hypothetical protein	34457.06
hctB	hctB Histone-like Protein 2	33501.69
CPn0331	CT082 hypothetical protein	33219.36
lcrH_1	lcrH_1 Low Calcium Response Protein H	27994.28
CPn0474	CT365 hypothetical protein	26041.66
CPn0443	CT005 hypothetical protein	25788.78
CPn0808	CT579 hypothetical protein	25269.04
groEL_1	groEL_1 Heat Shock Protein-60	25020.29
CPn0499	hypothetical protein	23849.93
yvyD	yvyD conserved hypothetical protein	23573.29
CPn0677	hypothetical protein	22986.91
DN9+10		22691.56
CPn0333	LnlB Protein	22416.03
CPn0369	CT058 hypothetical protein	21736.21
CPn0473	hypothetical protein	21652.78
pmp_19	pmp_19 polymorphic membrane protein A Family	19039.50
CPn0728	CHLPN 76 kDa Homolog_1 (CT622)	18847.95
CPn0809	CT578 hypothetical protein	18832.27
CPn0676	Similarity to CT695	17450.80
CPn0524	hypothetical protein	14635.06
CPn1016	CT858 hypothetical protein CPAF protease 1	14296.87
CPn0234	CT181 hypothetical protein	14240.84
CPn0588	CT469 hypothetical protein	14094.71
CPn0065	CT288 hypothetical protein	13905.70
ftsH	ftsH ATP-dependent zinc protease	13661.27
CPn0498	hypothetical protein	12893.22
CPn0370	CT058 hypothetical protein	12727.97
CPn0810	CT577 hypothetical protein	12334.96
CPn0875	CT734 hypothetical protein	11123.85
CPn0472	hypothetical protein	10901.14
yifF	yifF Cationic Amino Acid Transporter	10803.42
CPn0537	CT814.1 hypothetical protein	10288.45
CPn0667	hypothetical protein	10234.67
rpsD	rpsD Sigma-28/AviiG Family	9680.56
artJ	artJ Arginine Periplasmic Binding Protein	9553.60
CPn0329	Phospholipase D Superfamily	9017.13
yecA_3	yecA_3 Transport Permease	8825.55
CPn0572	CT456 hypothetical protein	8664.88
CPn0756	hypothetical protein FACS 4	8663.74
dagA_2	dagA_2 D-Alanine/Glycine Permease	8348.78
xerD	xerD Integrase/recombinase	8202.45
CPn0726	CT620 hypothetical protein	8175.83
pgi	pgi Glucose-6-P Isomerase	8055.55
thrS	thrS Threonyl-tRNA Synthetase	7987.45
CPn0720	CT659 hypothetical protein	7760.32
CPn0589	CT470 hypothetical protein	7758.09
CPn1004	CT847 hypothetical protein	7735.45
CPn0383	CT047 hypothetical protein	7292.22
accD	accD AcCoA Carboxylase/Transferase Beta	7137.78
CPn1005	CT848 hypothetical protein	7135.44
CPn0933	predicted disulfide bond isomerase	7119.73
clpP_1	clpP_1 ClP Protease	7029.07
CPn0964	hypothetical protein	6900.16
CPn0538	CT814 hypothetical protein	6882.66
pmp_15	pmp_15 Polymorphic Outer Membrane Protein B Family	6837.11
yscN	yscN Yop N (Flagellar-Type ATPase)	6752.80
yscN	yscN Yop N (Flagellar-Type ATPase)	6752.80
tlyC_2	tlyC_2 CBS Domains (Hemolysin homolog)	6692.52
dnaK	dnaK Heat Shock Protein-70	6667.15
secD/secF	secD/secF Protein Export Proteins SecD/SecF (fusion)	6650.81
vacB	vacB ribonuclease family	6535.30
pmp_2	pmp_2 Polymorphic Outer Membrane Protein G Family	6452.21
recA	recA RecA recombination protein	6369.57
arcD	arcD Arginine/Ornithine Antiporter	6360.19
dsbD	dsbD Thiodisulfide Interchange Protein	6356.90
pmp_6	pmp_6 Polymorphic Outer Membrane Protein G/I Family	6321.42

TABLE 3 (ii)

nqrA	nqrA Ubiquinone Oxidoreductase, Alpha	6319.39
gyrB_2	gyrB_2 DNA Gyrase Subunit B	6257.92
CPn0001	CT001 hypothetical protein	6197.90
mdhC	mdhC Malate Dehydrogenase	6185.13
lcrD	lcrD Low Calcium Response D	6173.29
ygcA	ygcA rRNA Methyltransferse	6160.83
dagA_1	dagA_1 D-Ala/Gly Permease	6131.94
CPn0105	CT016 hypothetical protein	6102.59
porB	ompB Outer Membrane Protein B (porin)	6032.94
ychF	ychF GTP Binding Protein	6024.47
CPn0705	CT668 hypothetical protein	6020.13
glyA	glyA Serine Hydroxymethyltransferase	5973.37
glyA	glyA Serine Hydroxymethyltransferase	5973.37
gpdA	gpdA Glycerol-3-P Dehydrogenase	5957.93
CPn0814	CT573 hypothetical protein	5949.06
pmp_12	pmp_12 Polymorphic Outer Membrane Protein (truncated) A/I Family	5943.77
CPn0507	CT421.1 hypothetical protein	5893.30
pmp_7	pmp_7 Polymorphic Outer Membrane Protein G Family	5881.74
yscC	yscC YopC/Gen Secretion Protein D	5874.56
glgB	glgB Glucan Branching Enzyme	5860.93
CPn1057	CT356 hypothetical protein	5800.47
CPn0925	CT779 hypothetical protein	5795.97
ysc3	ysc3 Yop Translocation 3	5794.03
CPn0512	CT425 hypothetical protein	5771.00
pmp_8	pmp_8 Polymorphic Outer Membrane Protein G Family	5763.89
gltX	gltX Glutamyl-tRNA Synthetase	5753.53
CPn1029	hypothetical protein	5743.24
rl3	rl3 L3 Ribosomal Protein	5696.59
fliN	fliN Flagellar Motor Switch Domain/YscQ family	5691.86
CPn0497	CT388 hypothetical protein	5674.36
tal	tal Transaldolase	5654.13
ldh	ldh Leucine Dehydrogenase	5644.61
fabI	fabI Enoyl-Acyl-Carrier Protein Reductase	5629.90
CPn0017		5609.25
yzeB	yzeB ABC transporter permease	5608.13
ftsY	ftsY Cell Division Protein FtsY	5606.91
pmp_21	pmp_21 Putative Outer Membrane Protein D Family	5604.24
CPn0727	CT619 hypothetical protein	5599.19
pmp_13	pmp_13 Polymorphic Outer Membrane Protein G Family	5583.84
clpC	clpC ClpC Protease	5577.87
CPn0107	CT058 hypothetical protein	5564.97
CPn0927	CHLPS 43 kDa protein homolog_2	5563.93
clpB	clpB Clp Protease ATPase	5558.20
dacF	dacF D-Ala-D-Ala Carboxypeptidase	5557.25
CPn0343	hypothetical protein	5542.02
ahpC	ahpC Thio-specific Antioxidant (TSA) Peroxidase	5528.77
proS	proS Prolyl tRNA Synthetase	5528.37
gp6D	gp6D CHLTR Plasmid Paralog	5527.11
yagE	yagE YagE family	5514.15
CPn0590	CT471 hypothetical protein	5498.93
CPn0822	CT565 hypothetical protein	5498.31
CPn0807	CT580 hypothetical protein	5497.50
CPn0929	CHLPS 43 kDa protein homolog_4	5497.45
dnlJ	dnlJ DNA Ligase	5470.60
folD	folD Methylene Tetrahydrofolate Dehydrogenase	5469.26
rl1	rl1 L1 Ribosomal Protein	5468.39
rl1	rl1 L1 Ribosomal Protein	5468.39
CPn0483	hypothetical protein	5434.74
clpP_2	clpP_2 CLP Protease Subunit	5418.10
rpoA	rpoA RNA Polymerase Alpha	5409.04
fabH	fabH Oxoacyl Carrier Protein Synthase III	5408.47
fusA	fusA Elongation Factor G	5403.23
CPn0220	hypothetical protein	5400.77
yebC	yebG YebC family hypothetical protein	5399.50
CPn1032	CT373 hypothetical protein	5396.91
gapA	gapA Glyceraldehyde-3-P Dehyrogenase	5377.30
dppD	dppD ABC ATPase Dipeptide Transport	5345.31
CPn0404	hypothetical protein	5322.62
pmp_14	pmp_14 Polymorphic Outer Membrane Protein H Family	5296.75
CPn0753	hypothetical protein	5290.01
CPn0462	hypothetical protein	5289.16
CPn0381	CT326 similarity	5286.39
CPn0374	CT056 hypothetical protein	5269.65

TABLE 3(iii)

CPn0799	hypothetical protein	5269.61
CPn0062	CT289 hypothetical protein	5249.79
alaS	alaS Alanyl tRNA Synthetase	5243.95
CPn0126	hypothetical protein	5240.36
rs4	rs4 S4 Ribosomal Protein	5239.14
CPn0623	CT504 hypothetical protein	5234.03
glgC	glgC Glucose-1-P Adenylyltransferase	5214.97
CPn0365	hypothetical protein	5195.21
CPn0458	hypothetical protein	5178.06
CPn0066	hypothetical protein	5136.84
CPn0742	CT635 hypothetical protein	5133.02
CPn0481	hypothetical protein	5118.37
sohB	sohB Protease	5106.44
CPn0675	CT696 hypothetical protein	5104.52
CPn0405	CT105 hypothetical protein	5095.20
lpxA	lpxA Acyl-Carrier UDP-GlcNAc O-Acyltransferase	5072.96
pmp_17.3	pmp_17.3 Polymorphic Outer Membrane Protein (Frame-shift with CPn0469)	5068.28
pepF	pepF Oligopeptidase	5065.00
ulpC	ulpC Hexosphosphate Transport	5064.37
CPn0705	CT671 hypothetical protein	5063.25
CPn0874	CT733 hypothetical protein	5047.64
pepA	pepA Leucyl Aminopeptidase A	5044.06
lipA	lipA Lipoate Synthetase	5008.60
CPn0514	CT427 hypothetical protein	5007.93
ribA/ribB	ribA/ribB GTP Cyclohydrolase & DHBP Synthase	5005.81
dut	dut dUTP Nucleotidohydrolase	4982.57
CPn0928	CHLPS 43 KDa protein homolog_3	4982.06
CPn0794	hypothetical protein FACS+ selected by Rita?	4965.51
CPn0798	hypothetical protein	4957.17
pnp	pnp Polyribonucleotide Nucleotidyltransferase	4954.15
fabG	fabG Oxoacyl (Carrier Protein) Reductase	4947.36
CPn0513	Fe-S oxidoreductase	4935.65
folP	folP Dihydropteroate Synthase	4934.67
yhhL	yhhL Decarboxylase Translocator	4929.02
pdhA/pdhB	pdhA/pdhB (pyruvate) Oxoisovalerate Dehydrogenase Alpha & Beta Fusion	4916.85
pyrH	pyrH UMP Kinase	4914.40
abcX	abcX ABC Transporter ATPase	4897.52
sucA	sucA Oxoglutarate Dehydrogenase	4895.62
yacB	yacB predicted phosphatase/kinase	4888.84
CPn0595	CT476 hypothetical protein	4877.72
CPn0014		4876.89
phnP	phnP Metal Dependent Hydrolase	4866.38
tyrP_2	tyrP_2 Tyrosine Transport	4861.62
CPn0559	CT444 hypothetical protein	4860.36
CPn0878	SET Domain protein	4858.94
ygo4	ygo4 Phosphate Permease	4856.29
tufA	tufA Elongation Factor Tu	4842.50
lpdA	lpdA Lipoamide Dehydrogenase	4831.92
CPn0397	PP2C phosphatase family	4831.19
ttiY	ttiY Glutamine Binding Protein	4828.17
phoH	phoH ATPase	4814.17
gltT	gltT Glutamate Symport	4807.24
lepA	lepA GTPase	4801.42
rho	rho Transcription Termination Factor	4800.55
ychM	ychM Sulfate Transporter	4793.79
pmp_16	pmp_16 Polymorphic Outer Membrane Protein B Family	4791.71
adt_2	adt_2 ADP/ATP Translocase	4773.25
CPn0484	hypothetical proline permease	4763.62
trxB	trxB Thioredoxin Reductase	4749.81
aroG	aroG Deoxyheptonate Aldolase	4747.65
ygaV	ygaV Hypothetical Protein	4722.09
cysS	cysS Cysteinyl tRNA Synthetase	4700.25
CPn0690	ABC Transporter Membrane Protein	4695.46
nqr2	nqr2 NADH (Ubiquinone) Dehydrogenase	4691.83
pmp_11	pmp_11 Polymorphic Outer Membrane Protein G Family	4690.71
CPn0035	CT339 hypothetical protein	4686.43
CPn0797	hypothetical protein FACS + selected by Rita?	4681.52
yaeT	yaeT Omp85 Analog	4680.61
greA	greA Transcription Elongation Factor	4676.81
cdsA	cdsA Phosphatidate Cytidylyltransferase	4673.11
CPn0803	CT584 hypothetical protein	4667.94
CPn0021	Predicted OMP [leader peptide]	4639.48
lsp	lsp Tail-Specific Protease	4639.10

TABLE 3(iv)

rl6	rl6 L6 Ribosomal Protein	4636.73
grpE	grpE HSP-70 Cofactor	4635.78
fabF	fabF Acyl Carrier Protein Synthase	4623.09
mgtE	mgtE Mg++ Transporter (CBS Domain)	4611.86
himD	himD Integration Host Factor Alpha	4600.92
CPn0422	CT273 hypothetical protein	4600.34
CPn0692	ABC Transporter	4591.16
CPn0415	CT266 hypothetical protein	4586.28
aspC	aspC Aspartate Aminotransferase	4534.97
dcd	dcd dCTP Deaminase	4533.88
CPn0010.1	frame-shift with CPn0010	4530.44
atoC	atoC 2-Component Regulator	4528.67
gatB	gatB (Pet112) Glu-tRNA-Gln Amidotransferase (B Subunit)	4510.59
CPn0807	CT560 hypothetical protein	4507.66
yceA	yceA YceA hypothetical protein	4491.46
metG	metG Methionyl-tRNA Synthetase	4490.29
atpA	atpA ATP Synthase Subunit A	4483.96
CPn1054	hypothetical protein	4483.87
rl5	rl5 L5 Ribosomal Protein	4482.91
xseA	xseA Exodeoxyribonuclease VII	4469.21
CPn0859	CT718 hypothetical protein	4460.54
CPn0449	pmp_10 (frameshift)	4458.00
nqr5	nqr5 NADH (Ubiquinone) Reductase 5	4453.91
CPn0041	hypothetical protein	4453.05
accA	accA AcCoA Carboxylase/Transferase Alpha	4443.85
oppA_2	oppA_2 Oligopeptide Binding Protein	4442.36
valS	valS Valyl tRNA Synthetase	4442.14
CPn0456	hypothetical protein	4436.51
CPn0681	CT691 hypothetical protein	4426.13
dnaA_2	dnaA_2 Replication Initiation Factor	4424.21
CPn0007	hypothetical protein	4423.42
yscT	yscT YopT Translocation T	4421.69
rs3	rs3 S3 Ribosomal Protein	4418.77

Chlamydia Further Filing/
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TABLE 3(v)-(xi)

CPn	CT	Protein Function	MRNA levels	Stage in the Chlamydial developmental cycle	Reference *(see legend)*	EB Surface exposed	RB secreted
	CT081	CHLTR T2 protein		Very Late	Nicholson		
0557	CT443	OmcB	43304.22	Late Gene	Belland Shaw	EB Surface exposed	
0558	**CT444**	**OmcA**	**42530.16**	**Late Gene**	**Belland**	**Yes FACS pos In vivo protective with CT467**	
0695	CT681	MOMP		Mid Late II		FACS positive	
0384	CT046	hctB		Late Gene	Belland		
0331	**CT082**	**Hypothetical**	**33219.36**	**Late**	**Nicholson**	**FACS positive**	
0811	CT576	LcrH		Late Gene	Belland		
0474	CT365	Hypothetical		Immediate/Early	Belland		
0443	CT005						
0808	CT579	Hypothetical		Late Gene	Belland		Type III secretion cluster (WO 02/082091)
0134	CT110	GroEL Heat shock protein (Hsp-60)		Immediate Early gene Midcycle Midlate I	(see Table 1 of Belland et al (2003) Nicholson	FACS positive	
0499		Cpn hypothetical protein					
		Yyd conserved hypothetical protein					
		CPn specific protein					
0333	CT080	ltuB		Late Gene	Belland		
0369	CT058						
0539	**CT412**	**Pmp19**	**19039.90**			**FACS positive**	
0728	CT622						
0809	CT578			Late Gene	Belland		
0676	CT695						
1016	CT858	predicted Protease containing IRBP					Secreted Protein (WO 02/082091) Epitope

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0234	CT181	Hypothetical				FACS positive	
0588	CT469	Hypothetical					
0065	CT288						
0998	CT841						Epitope (WO 02/082091)
0369	CT058						
0810	CT577	Hypothetical					Type III secretion cluster (WO 02/082091)
0875	CT734	Hypothetical		Immediate Early	Belland		Secreted Protein (WO 02/082091)
0127	CT034						
0538	CT814			Late	Belland		
0482	CT381	ArtJ				FACS positive	
0329	CT154-158	CT157 Phospolipase D Superfamily				FACS positive	
0572	**CT456**	**Hypothetical (now TarP)**	8664.88			FACS positive	**CT456 is secreted from Chlamydia by a Type III secretion system (TTSS) (Clifton et al 2004) and is translocated into the cytoplasm of the host cell. New function assigned = Tarp = translocated actin-recruiting phosphoprotein** **CT456 gene is transcribed from mid to late cycle in the Chlamydia developmental cycle** CT456 may vary across the serovars because it has tandem repeats
0876	CT735						

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1024	CT864						
0726	CT620						
1025	CT378						
0482	CT581						
0720	CT659			Late	Belland		
0589	CT470						
1004	CT847			Late	Belland		
0383	CT047						
0058	CT293						
1005	CT848						
0933	CT783	predicted disulfide bond isomerase					Secreted Protein (likely to be a Type III secretion protein) (WO 02/082091)
0520	CT431						
0538	CT814						
0466	CT869	PmpE				FACS positive	
0707	CT669	yscN					Type III secretion protein
0707	CT669	yscN		Mid-cycle	Shaw		
0503	**CT396**	**DnaK**	**6667.15**	**Late**	**Nicholson**	**FACS positive**	
0564	CT448						
0504	CT397						
0453	CT871	PmpG		Late	Belland	FACS positive	
0762	CT650	recA					Secreted Protein (WO 02/082091)
1031	CT374						
0786	CT595						
0743	CT634						
0715	CT661						
0001	CT001			Late	Nicholson		
1028	CT376						
0323	CT090						
0885	CT742						
0876	CT735						
0105	CT016	Hypothetical				FACS positive	**Secreted Protein (see WO 02/48185), pg 36 (ie secreted by a Type III apparatus)**
0854	CT713	PorB		Midlate II	Nicholson	FACS positive	

Chlamydia Further Filing/
Microarray Expressed Gene Table
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TABLE 3(v)-(xi)

0321	CT092						
0708	CT668	Hypothetical					Secreted Protein (WO 02/082091)
0520	CT432						
0521	CT432						
0855	CT714						
0814	CT573						
0506	CT421						
0702	CT674						
0475	CT866						
1057	CT356						
0925	CT779						
0828	CT559	YscJ				FACS positive	
0512	CT425						
0560	CT445						
0647	CT528						
0704	CT672			mid late I	Nicholson		
0497	CT388						
0083	CT313						
0919	CT773						
0406	CT104						
1012	CT854						
0880	CT739						
0963	CT812	PmpD				FACS positive	
0727	CT619						
0437	CT286	ClpC					Secreted Protein (WO 02/082091)
0369	CT058						
0134	CT110	Hsp-60 (omp2) Chaperonin		Immediate Early gene Midcycle Midlate I	(see Table 1 of Belland et al (2003) Nicholson	FACS positive	
0672	CT551						
0778	CT603						
0500	CT393						
0804	CT583						
0590	CT471						
0822	CT565						
0807	CT580						
0149	CT146						
0335	CT078						

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TABLE 3(y)-(xl)

0078	CT318						
0078	CT318						
0520	CT431						
0626	CT507						
0298	CT239			Mid late	Nicholson		
0550	CT437						
0573	CT457						
1032	CT373						
0624	CT505						
0682	CT690						
0381	CT326						
0374	CT056						
0062	CT289						
0598	CT479						
0733	CT626						
0623	CT504						
0607	CT489						
0742	CT635	Hypothetical				FACS Positive	
0613	CT494			mid late II	Nicholson		
0675	CT696						
00405	CT105						
0650	CT531						
0136	CT112						
0665	CT544						
0705	CT671	Hypothetical				FACS Positive	**WO 02/48185 CT671 = secreted protein, pg 36 (ie secreted by a Type III apparatus) Epitope**
0874	CT733						
0385	**CT045**	**PepA**	**5044.06**	**Mid late I**	**Nicholson**	**FACS Positive**	
0832	CT558						
0514	CT427						
0904	**CT761**	**MurG**	**5005.81**	**See biogenome paper 2004 – MurG was consistently selected across the Serovars**		FACS Positive	
0059	CT292	dut					Secreted Protein (WO 02/082091)

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0999	CT842						
0296	CT237						
0513	CT426						
0758	CT613						
0207	CT204						
0304	CT245						
0698	CT678						
0191	CT130						
0378	CT054						
0611	CT492						
0584	**CT467**	**AtoS**	**4877.72**	**Cross-reactivity between CT and CPn strains Ie CT467 and its CPn homologue are *neutralising* for their own species but are also cross-protective**		**FACS Positive**	
0479	CT380						
0970	CT818						
0558	**CT444**	**OmcA**		**Late gene (see Table 1 of Belland et al (2003)**		**FACS Positive** **In vivo protective effect with CT444 and CT467**	
0878	CT737						
0680	CT692						
0074	CT322						
0833	CT557						
0793	CT588						
0604	CT486						
0106	CT015						
0528	CT401						
0359	CT064						
0610	CT491			Mid late II	Nicholson		
1014	CT856						
0466	CT869?						
0351	CT065	ADP/ATP Transclocase					
0314	CT099						
0484	CT382						
0932	CT782						
0690	CT686						
0427	CT278						
0453	CT871	pmpG		*Late gene*	(see Table 1 of Belland et	FACS Positive	

Chlamydia Further Filing/
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					al (2003)		
0035	CT339						
0300	CT241						
0741	CT636						
0567	CT451						
0803	CT584						
0555	CT441						
0633	CT514						
0502	CT395						
0916	CT770						
0286	CT194						
0416	CT267						
0422	CT273						
0692	CT684						
0415	CT266	Hypothetical				FACS Positive	
0495	CT390						
0392	CT039						
0586	CT468						
0004	CT004						
0827	CT560	Hypothetical protein					Type III secretion cluster (WO 02/082091)
0734	CT627						
0122	CT032						
0657	CT537						
0635	CT516						
1062	CT329						
0859	CT718						
0430	CT281						
0414	CT265	AccA					Secreted Protein (WO 02/082091)
0599	CT480	Oligopeptide binding lipoprotein (Oppa)		Immediate early gene	(see Table 1 Belland et al 2003)	FACS Positive	
0094	CT302						
0681	CT691	Hypothetical					Secreted Protein (WO 02/082091) Epitope
0309	CT250						
0823	CT564						
0641	CT522						

Table 4. *C. pneumoniae* selected peptides: protein sources and HLA-A2 stabilization assay

Peptide	Sequence	CPn [a]	Protein	Group [b]	Score [c]	Net MFI [d]
HepB	^{125}TAFHQTLQD^{133}		Hepatitis B virus envelope antigen		======	14.0±24.4
GAG	^{77}SLYNTVATL^{85}		HIV-1 gag		157.22	92.4±23.8
IMa	^{58}GILGFVFTL^{66}		Influenza virus matrix M1		550.92	63.1±18.1
CH1	^{315}QLLDEGKEL^{323}	0322	*Yop proteins translocation protein U*	Type III	324.06	74.0±22.6
CH2	^{433}LLNEVPYV^{441}	0323	Low calcium response protein D	Type III	5534.14	140.5±36.1
CH3	^{343}VLNLFFSAL^{351}	0324	Low calcium response protein E	Type III	262.20	40.1±23.1
CH4	^{7}QLLESLAPL^{15}	0325	Secretion chaperone	Type III	745.35	120.1±25.2
CH5	^{271}SILELLQFV^{279}	0702	Probable Yop proteins translocation protein C	Type III	1835.22	85.5±34.4
CH6	^{79}YLLEEIYTV^{87}	0811	Low calcium response protein H	Type III	11162.99	148.5±38.9
CH7	^{83}YMDNNLFYV^{91}	0823	Yop proteins translocation protein T	Type III	6781.36	164.1±24.3
CH8	^{254}FLTLAWWFI^{262}	0823	Yop proteins translocation protein T	Type III	3365.36	144.1±22.1
CH10	^{214}GLTEEIDYV^{222}	0828	Yop proteins translocation protein J	Type III	1767.58	144.0±37.9
CH12	^{79}WLVFFNPFV^{87}	1021	Low calcium response locus protein H	Type III	6686.72	50.1±22.2
CH13	^{69}YVFDRILKV^{77}	0695	Outer membrane protein A	Ch spec	976.76	139.0±36.7
CH14	^{406}VMLIFEKKV^{414}	0415	CT2 66 hypothetical protein	Cpn spec	1200.64	74.1±20.2
CH15	^{1270}YLTSYSPYV^{1278}	0444	Polymorphic outer membrane protein G/I family	Pmp	1759.66	138.1±23.5
CH16	^{1530}VQLAYVFDV^{1538}	0963	*Putative outer membrane protein D family*	Pmp	591.70	48.1±19.1
CH17	^{308}ILQEAEQMV^{316}	0728	76 kDa homolog_1	Ch spec	484.77	202.1±24.2
CH18	^{71}IALLVIFFV^{79}	0186	Similar to CT119 IncA	Ch spec	445.80	46.1±22.3
CH19	^{1327}LLLTLGYAV^{1335}	0444	Polymorphic outer membrane protein G/I family	Pmp	437.48	56.1±21.6
CH20	^{146}ALMLLNNYV^{154}	0005	Polymorphic outer membrane protein G family	Pmp	1415.38	142.5±38.6
CH21	^{614}TLWGSFVDV^{622}	0447	Polymorphic outer membrane protein G/I family	Pmp	1096.83	121.1±18.0
CH22	^{1566}WLFDLRFSV^{1574}	0540	Polymorphic membrane protein B family	Pmp	28150.17	68.5±11.0
CH24	^{490}LIQETLLFV^{498}	0021	Predicted OMP	Ch spec	843.21	105.1±20.8
CH28	^{45}RLLEIIWGV^{53}	0062	CHLPS 43 kDa protein homolog_1	Cpn spec	18200.54	99.5±15.0
CH29	^{288}YLMQKLQNV^{296}	0791	CT 590 hypothetical protein	Ch spec	2722.68	108.5±12.1
CH30	^{520}FLQRGESFV^{528}	0792	CT 589 hypothetical protein	Ch spec	759.66	105.1±8.1
CH31	^{401}WLLRDDWLL^{409}	0009	hypothetical	Cpn spec	2726.91	101.1±16.3
CH32	^{187}KLWEWLGYL^{195}	0041	hypothetical	Cpn spec	4184.21	72.1±12.0
CH33	^{68}LLMLAISLV^{75}	0131	hypothetical	Cpn spec	1006.20	18.0±1.4
CH34	^{201}KLLKDHFDL^{209}	0132	hypothetical	Cpn spec	1604.53	85.1±4.9
CH35	^{56}ILSFLPWLV^{64}	0169	hypothetical	Cpn spec	886.78	90.6±5.7
CH36	^{149}LLLIFNNYL^{157}	0170	hypothetical	Ch spec	2808.32	41.0±19.8
CH37	^{126}YLLDFRWPL^{134}	0210	hypothetical	Cpn spec	42485.26	97.1±17.7
CH38	^{374}NLLKRWQFV^{382}	0352	hypothetical	Cpn spec	2406.15	64.1±13.4
CH39	^{378}FLLRHLSSV^{386}	0355	hypothetical	Cpn spec	2722.68	88.1±6.4
CH41	^{162}KLSEQLEAL^{170}	0186	Similar to CT119 IncA	Ch spec	345.48	51.6±27.6
CH42	^{214}KVLLGQEWV^{222}	0186	Similar to CT119 IncA	Ch spec	212.39	16.0±37.5
CH43	^{315}NLAEQVTAL^{323}	0186	Similar to CT119 IncA	Ch spec	201.44	71.6±34.6
CH44	^{123}YVVGFIIFL^{131}	0323	Low calcium response protein D	Type III	413.32	25.0±16.3
CH45	^{32}WMMGVVLMI^{40}	0323	Low calcium response protein D	Type III	294.95	8.1±18.4
CH46	^{56}NLSISVFLL^{64}	0323	Low calcium response protein D	Type III	284.97	18.0±26.2
CH47	^{110}VIQAFGDFV^{118}	0323	Low calcium response protein D	Type III	166.49	23.0±32.5
CH48	^{635}YLALDPDSV^{643}	0323	Low calcium response protein D	Type III	156.77	74.6±34.4
CH49	^{149}KMSHFQQAL^{157}	0415	CT2 66 hypothetical protein	Cpn spec	205.19	29.0±33.9
CH50	^{1187}SLCAQSSYV^{1195}	0444	Polymorphic outer membrane protein G/I family	Pmp	382.53	45.1±22.6
CH51	^{1360}NLSRQAFFA^{1368}	0444	Polymorphic outer membrane protein G/I family	Pmp	158.47	25.0±34.5
CH52	^{678}SLLEEHPVV^{686}	0963	Putative outer membrane protein D family	Pmp	432.59	43.6±21.9
CH53	^{1302}NLWSHYTDL^{1310}	0963	Putative outer membrane protein D family	Pmp	265.96	1.6±24.7
CH54	^{377}ALWKENQAL^{385}	0963	*Putative outer membrane protein D family*	Pmp	177.30	45.6±21.9
CH55	^{568}ALWGHNVLL^{576}	0963	Putative outer membrane protein D family	Pmp	177.30	47.6±29.0
CH56	^{333}NLAGGILSV^{341}	0963	Putative outer membrane protein D family	Pmp	159.97	29.6±29.0
CH57	^{86}FVSKFWFSL^{94}	1021	*Low calcium response locus protein H*	Type III	322.16	16.0±0.1
CH58	^{73}SITVFRWLV^{81}	1021	Low calcium response locus protein H	Type III	272.55	30.0±20.5
CH59	^{58}YLIVFVLTI^{66}	0131	hypothetical	Cpn spec	419.44	29.0±10.6
CH60	^{42}VMLFIGLGV^{50}	0131	hypothetical	Cpn spec	315.95	28.0±2.8
CH61	^{76}VLFLLIRSV^{84}	0131	hypothetical	Cpn spec	201.24	14.0±2.8
CH62	^{397}FLFQLGMQI^{405}	0415	CT2 66 hypothetical protein	Ch spec	177.56	30.0±12.0

[a] Gene sequence designation as annotated from the genome sequence of Cpn strain CWL029 (http://chlamydia-www.berkeley.edu:4231)

[b] Type III: type III secretion system; Ch and Cpn spec: Chlamydia and C. pneumoniae specific; Pmp: Polymorphic membrane protein

[c] Calculated using the BIMAS algorithm

[d] Mean Fluorescence Intensity of cells with peptide - M Mean Fluorescence Intensity of cells without peptide ± Standard Deviation calculated on three experiments

Table 5. ELISpot assay with $CD8^+$ T cells from DNA immunized HLA-A2 transgenic mice

Protein	Gene	Peptide	SFC [a]
Hypothetical	CPn 0131	medium	13
		HepB	47
		CH 33	33
		CH 59	53
		CH 60	80
		CH 61	40
Hypothetical	CPn 0210	medium	7
		HepB	13
		CH 37	120
LCR Protein D	CPn 0323	medium	27
		HepB	27
		CH 2	93
		CH 44	80
		CH 45	87
		CH 46	40
		CH 47	80
		CH 48	60
CHLPS 43 kDa	CPn 0062	medium	33
		HepB	27
		CH 28	93
OMP A	CPn 0695	medium	13
		HepB	33
		CH 13	727
LCR Protein H	CPn 0811	medium	13
		HepB	27
		CH 6	213
Yop pt protein T	CPn 0823	medium	7
		HepB	47
		CH 7	493
		CH 8	53
Yop pt protein J	CPn 0828	medium	20
		HepB	60
		CH 10	247

a) SFC = Spot Forming Colonies/10^6 CD8 cells

Table 6. IFN-γ production from splenocytes of DNA immunized HLA-A2 transgenic and non transgenic mice

			Ex vivo RFI [a]		stTCLs[b] RFI [a]	
Protein	Gene	Peptide	$A2^{-}$ [c]	$A2^{+}$ [c]	$A2^{-}$ [c]	$A2^{+}$ [c]
LCR Protein D	CPn 0323	CH 2	0,05	1,11	0,79	2,57
		CH 44	3,30	1,78	0,73	6,86
		CH 45	1,00	1,56	0,47	4,71
		CH 46	0,90	1,44	0,41	9,00
		CH 47	1,00	1,78	1,17	1,14
		CH 48	1,30	1,67	0,11	1,29
		CD3+CD28	134,00	90,55		
OMP A	CPn 0695	CH 13	3,29	2,54	23,42	209,81
		CD3 + CD28	248,71	73,23		
LCR Protein H	CPn 0811	CH 6	1,00	4,58	1,53	31,56
		CD3 + CD28	290,83	96,10		
Yop pt Protein T	CPn 0823	CH 7	1,20	5,20	11,69	94,57
		CH 8	2,00	1,60	16,81	28,21
		CD3 + CD28	247,60	91,00		

[a] Relative Fold Increase: ratio between the percentage of $IFN\text{-}\gamma^{+}/CD8^{+}$ cells obtained with the tested peptide (or the CD3/CD28 co-stimulus) and the HepB negative control peptide

[b] Short term T cell lines

[c] HLA-A2 non transgenic and transgenic mice